# CENTER FOR DRUG EVALUATION AND RESEARCH

***APPLICATION NUMBER:***

# 201280Orig1s000

# <u>PHARMACOLOGY REVIEW(S)</u>

**Tertiary Pharmacology/Toxicology Review**

**From**: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
**NDA:** 201280
**Agency receipt date:** July 2, 2010
**Drug:** linagliptin
**Sponsor:** Boehringer Ingelheim Pharmaceuticals, Inc.
**Indication:** Type 2 Diabetes Mellitus treatment

**Reviewing Division:** Division of Metabolism and Endocrinology Products

**Introductory Comments:** The pharm/tox reviewer and team leader concluded that the nonclinical data support approval of linagliptin for the indication listed above.

Linagliptin was not teratogenic in rats or rabbits at doses providing large margins of exposure compared to humans. Some findings such as flat/thickened ribs were noted at maternally toxic doses. These doses produced exposures that were much higher than those achieved in humans (1000 fold and higher). The reviewer and supervisor recommend that linagliptin be labeled pregnancy category B. This is consistent with other members of this pharmacologic class.

Carcinogenicity studies of linagliptin were conducted in rats and mice. The Executive Carcinogenicity Assessment Committee concurred that the only apparent drug-related finding was lymphomas in female mice at the highest dose. The dose associated with lymphomas produced an exposure in mice that was much greater (>200 fold) than the exposure in humans at the maximum recommended human dose.

**Conclusions:**
I agree with the division pharm/tox conclusion that linagliptin can be approved from the pharm/tox perspective. The lymphoma finding, while apparently drug-related in the mouse carcinogenicity study, is unlikely to present a significant risk to patients given the large difference in exposure between mice and humans. I agree with the labeling as suggested by the pharm/tox reviewer and supervisor.

---------------------------------------------------------------------------------------------------------

**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**

---------------------------------------------------------------------------------------------------------

/s/

----------------------------------------------------

PAUL C BROWN
03/24/2011

DEPARTMENT OF HEALTH & HUMAN SERVICES Food and Drug Administration

Memorandum

**Pharmacology/Toxicology**
**Center for Drug Evaluation and Research**
**Division of Metabolic & Endocrine Products**

## NDA SECONDARY REVIEW

| | |
|---|---|
| Date: | 10 March 2011 |
| NDA # | 201280 |
| Sponsor: | Boehringer Ingelheim Pharm. Inc |
| Drug: | Linagliptin |
| Primary Reviewer: | David Carlson, Ph.D. |
| Secondary Reviewer: | Todd Bourcier, Ph.D. |

Boehringer Ingelheim is seeking marketing approval for linagliptin as a treatment option for Type 2 diabetes. Linagliptin is a member of the dipeptidyl peptidase-4 (DPP4) inhibitor class of compounds whose primary mode of action consists of extending the half-life of the incretin GLP-1, thereby enhancing glucose-induced release of insulin from pancreatic beta cells. Among many in development, two DPP4 inhibitors are approved for prescription use in the US (sitagliptin and saxagliptin) and one is approved for non-US markets (vildagliptin).

Dr. David Carlson, the primary reviewer, concludes that the pharmacology and toxicology data support approval of linagliptin (5 mg q.d.) without the need for non-clinical PMRs or PMCs.
*I concur with Dr. Carlson's assessment.*

Dr. Carlson's decision is based on the relatively benign toxicological profile of linagliptin in rats, dogs, mice, and monkeys over a considerable range of drug exposure relative to the clinical dose of 5mg/day. Very high exposure (≥ 50 fold the clinical dose) was associated with target organ toxicity in animals, which included adverse effects in the kidney, lung, liver, stomach, and male reproductive organs. Several of these toxicities (e.g., lung phospholipidosis, renal tubular and hepatocellular damage) have been reported with other DPP4 inhibitors indicating a class-related toxicological mechanism, but these are not considered indicative of risk to human subjects *when observed at high multiples* of the therapeutic dose. Cutaneous toxicity in monkeys associated with some DPP4 inhibitors was not observed with linagliptin, despite exposure to high doses for 12 months duration. Given the large exposure multiples, even moderate increases of drug exposure in susceptible patients would present a negligible risk of reproducing animal toxicities in human subjects at clinically relevant doses.

Pancreatitis has arisen as a safety concern for GLP1 targeted therapeutics, including the DPP4 inhibitors. Linagliptin did not cause histological changes in the pancreas of animals indicative of pancreatitis or pancreatic injury, despite long-term exposure to very high doses of drug. I agree with Dr. Carlson's assessment that a limitation to extrapolating these studies to the intended diabetic clinical population is that toxicity studies are conducted in normoglycemic healthy animals.

Findings in the carcinogenicity assessment were similarly observed only at high drug levels and present negligible clinical risk. The executive carcinogenesis assessment committee concluded that the 2 yr study in mice was positive for lymphoma in females at a dose approximating 287-times the clinical dose. No drug-related tumors were observed in male mice or in rats of either sex. Linaglitpin and its major metabolites were not genotoxic when tested in an array of in vitro and in vivo assays. These findings are described in Section 13 of the drug label.

Findings from the reproductive toxicology studies did not reveal relevant toxicities without also producing signs of maternal toxicity. Prenatal and postnatal exposure to drug in animals did not result in morphological, functional, behavioral, or reproductive toxicity at doses up to 49-times the clinical dose. These studies support the designation of category 'B' in section 8.1 of the drug label.

---

**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**

---

/s/

---

TODD M BOURCIER
03/10/2011
pharm/tox supports NDA approval

**DEPARTMENT OF HEALTH AND HUMAN SERVICES**
**PUBLIC HEALTH SERVICE**
**FOOD AND DRUG ADMINISTRATION**
**CENTER FOR DRUG EVALUATION AND RESEARCH**

# PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

| | |
|---|---|
| Application number: | 201280 |
| Supporting document/s: | Original Submission (and updates) |
| Applicant's letter date (CDER Stamp Date): | 2 July, 2010 (7/2/10) |
| Product: | Linagliptin tablets (Tradjenta – proposed name) |
| Indication: | Type 2 Diabetes Mellitus treatment |
| Applicant: | Boehringer Ingelheim Pharmaceuticals, Inc. |
| Review Division: | Metabolism and Endocrinology Products |
| Reviewer: | David B. Carlson, Ph.D. |
| Supervisor/Team Leader: | Todd Bourcier, Ph.D. |
| Division Director: | Mary Parks, M.D. |
| Project Manager: | Raymond Chiang. M.S. |
| Review Completion Date: | 4 March, 2011 |

*Template Version: September 1, 2010*

**Disclaimer**

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 201280 are owned by Boehringer Ingelheim Pharmaceuticals Inc. (BIPI) or are data for which BIPI has obtained a written right of reference. Any information or data necessary for approval of NDA 201280 that BIPI does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 201280.

**Review Notes and Abbreviations/Key**

Some of the sponsor's tables and figures from the electronic NDA submission have been included and cited in this review. All drug-related trends are discussed in relation to concurrent vehicle control groups in each study unless otherwise noted. Vehicle for oral gavage administration was 0.5% hydroxyethylcellulose (Natrosol® 250 HX) unless otherwise noted. Common animal strains were used and abbreviated by common animal name, unless noted, as follows: Wistar Han rat, CD-1 mouse, Beagle dog, Cynomolgus monkey, New Zealand White rabbit. Discussions and conclusions in this NDA review represent the opinions of this pharmacology/toxicology reviewer and incorporate conclusions from nonclinical studies reviewed under IND 70,963, some of which are summarized throughout this review[1].

**Key:** Dipeptidyl peptidases – DPP4 (aka DPP-4), DPP2, DPP8, DPP9; USP (United States Pharmacopeia); NF (National Formulary); Dosing groups – LD (low dose), MD (mid dose), LMD (low mid dose), HMD (high mid dose), HD (high dose); mg/kg (mg/kg/day); once daily dosing (QD), twice daily dosing (BID); MRHD (maximum recommended human dose); NOAEL (no observed adverse effect level); LOAEL (lowest observed adverse effect level); statistically significant (ss); not statistically significant (nss); PD (pharmacodynamic), PK (pharmacokinetic), TK (toxicokinetic); BW (body weight); AUC (integrated 'area under the curve'); GD (gestation day); LD (lactation day); central nervous system (CNS), peripheral nervous system (PNS); OGTT (oral glucose tolerance test)

[1] IND 70,963 pharmacology/toxicology reviews by S. Xiao, A. Parola, and D. Carlson.

# TABLE OF CONTENTS

## Table of Tables

## Table of Figures

# 1 Executive Summary

## 1.1 Introduction

The proposed linagliptin film-coated tablet was submitted in accordance with 21 USC 505(b)(1) for treatment of type 2 diabetes mellitus as an adjunct to diet and exercise. A comprehensive battery of nonclinical studies were conducted to support development of linagliptin for chronic use. Linagliptin is the fifth DPP4 inhibitor application submitted for FDA review for approval to treat type 2 diabetes. Two DPP4 inhibitors have been approved for diabetes treatment and the toxicity profile of linagliptin compares favorably to the listed drugs. All pivotal studies with linagliptin were conducted in compliance with current GLP standards.

## 1.2 Brief Discussion of Nonclinical Findings

All pivotal nonclinical studies were conducted using oral administration of drug, which is the clinical exposure route, and in accordance with US FDA GLP regulations (21CFR58) as stated by Sponsor and confirmed by this reviewer[2]. Toxicity studies in healthy, non-diabetic animals were sufficient to identify NOAEL exposures for comparison to clinical exposure.

Safety margins to expected human exposure were estimated using $C_{max}$ = 11 nM and $AUC_{0-24}$ = 158 nM*h plasma exposure in diabetic subjects at the proposed maximum recommended human dose (MRHD) of 5 mg linagliptin[3]. Linagliptin (BI 1356 BS) is not highly metabolized and is excreted largely unchanged. A single major human metabolite, CD 1790 ($AUC_{0-24}$ = 20 nM*h; 13% linagliptin exposure), was monitored and qualified in nonclinical studies. CD 1750 is the racemic mixture of the pure R-enantiomer CD 1790 and the minor S-enantiomer metabolite CD1789. CD 1750 was often monitored and described as interchangeable with CD 1790 based on limited [redacted] (b) (4) impurities and limited chiral conversion of R-enantiomeric parent drug or metabolites.

Linagliptin is an orally active dipeptidyl peptidase IV (DPP4) inhibitor. DPP4 is typically expressed on the cell surface of a wide variety of tissues, including kidney, liver, intestine, lymphocytes, and vascular endothelial cells. DPP4 is also present and active in plasma, which is the therapeutic target of linagliptin. DPP4 cleaves polypeptides at [redacted] (b) (4) residues at the N-terminus. DPP4-mediated cleavage regulates a variety of peptides including peptide hormones, neuropeptides, and chemokines. Regulation of incretin hormones in the gut, glucagon-like peptide 1

---

2 Pivotal studies were conducted in accordance with OECD and/or member states GLP principles, which are acceptable under US agreements

3 Steady state plasma exposures measured in multiple dose PK trial 1218.2 and consistent with mean population PK from pivotal clinical trials

(GLP-1) and glucose-dependent insulinotropic peptide (GIP), appears to be the primary role of DPP4 in regulating postprandial glucose. The incretins, in combination but with GLP-1 playing a key role, increase insulin secretion, enhance insulin receptor sensitivity, and decrease hepatic glucose, gastric emptying, and food intake. DPP4 inhibition prevents the natural rapid breakdown of GLP-1 and GIP after their postprandial expression.

Linagliptin pharmacology was assessed in a variety of *in vitro* and *in vivo* animal models to investigate DPP4 inhibitory activity and effects on blood glucose. Linagliptin and other DPP4 inhibitors have been shown to reduce blood sugar and glycated hemoglobin ($HbA1_c$) *in vivo* in healthy and diabetic animal models and in diabetic patients. Two DPP4 inhibitors, sitagliptin (Januvia™) and saxagliptin (Onglyza®) are currently marketed in the U.S. and globally for treatment of type 2 diabetes. A third DPP4 inhibitor, vildagliptin, is currently marketed in various countries outside the U.S. In addition, exenatide and liraglutide are DPP4-resistant mimetics/analogs of GLP-1 that are approved for treatment of type 2 diabetes in the U.S. Linagliptin is proposed to be used as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes.

Linagliptin achieves efficacy at relatively low drug concentrations despite having comparable inhibitory potency to other DPP4 inhibitors. This may be in part a result of linagliptin exhibiting high affinity binding to DPP4 in plasma and tissues which results in a very long plasma terminal half-life of up to 100 h in animals and humans. Linagliptin binds to and saturates DPP4 expressed in the kidney, liver, lung, and other DPP4-containing tissues. Drug is released slowly over several days from these DPP4-expressing tissues. Studies with DPP4-deficient and DPP4 knockout rodents confirmed the absence of tissue accumulation in animals lacking tissue DPP4. There is no apparent toxicity associated with accumulation of linagliptin in kidney, liver or other DPP4-expressing tissues. In the blood and at therapeutic drug concentrations (~10nM), linagliptin binds to and saturates plasma DPP4 with high affinity, while excess drug binds to other plasma proteins with lower affinity. As drug concentration increases further, as it did in the toxicology studies, linagliptin continues to bind with low affinity to other plasma proteins. As a result, the free fraction of drug continues to increase as the drug concentration increases. The sustained and high affinity binding of linagliptin to the DPP4 enzyme both in tissues and in the blood likely contribute to achieving efficacy at drug concentrations only 2 to 3-fold higher than the *in vitro* IC50 inhibitory potency for DPP4 activity (3.6nM).

Linagliptin showed high selectivity for inhibiting DPP4, with >10,000-fold selectivity for DPP4 compared to the closely related cytoplasmic dipeptidyl peptidases DPP8 and DPP9, which are differentially expressed in skeletal muscle, heart, liver, and activated T-cells. While the physiological role of DPP8 and DPP9 are still unknown, inhibition of DPP8/9 have been associated with animal toxicity including alopecia, histopathological changes in multiples organs, gastrointestinal toxicity, and blood and immune system effects (thrombocytopenia, reticulocytopenia, enlarged spleen) which may be mediated by inhibition of T-cell proliferation (Lankas GR et al., Diabetes (2005) 54(10):2988).

Skin, immune, and GI-related toxicity have been observed with some DPP4 inhibitors and, based on the similar toxicity profile with selective DPP8 and/or DPP9 inhibition, some toxicity attributed to DPP4 inhibition may be due to off-target inhibition of DPP8/9. Specifically, edema and necrotic skin lesions have been seen with several DPP4 inhibitors, which may be due to off-target inhibition of DPP8 and/or DPP9. No skin lesions were seen in monkeys or other species treated with linagliptin.

The high selectivity of linagliptin for DPP4 predicts a limited risk for toxicities related to inhibition of other 'DASH' family enzymes[4]. Linagliptin was generally well tolerated in healthy and diabetic animals. Irreversible and/or non-monitorable toxicity typically occurred only at very high exposure multiples (>90-times the MRHD). As noted above, linagliptin did not cause skin lesions in monkeys, which has been a toxicity of concern for some drugs in the class. Linagliptin produced a pseudoallergy-type, hypersensitivity response in dogs and minipigs after oral dosing and in monkeys only after very high *iv* exposures (>600-times clinical exposure). The toxicity presented as facial flushing/reddening and edema, but was tolerable even at high doses of linagliptin. The pseudoallergy reaction has been shown to involve systemic histamine release but there was no evidence of an IgE-mediated allergic response that could increase the risk of anaphylaxis. Investigative studies showed linagliptin was not an irritant or hemolytic.

Target organs were identified only at very high exposure multiples with NOAELs >30-times clinical exposure in chronic toxicity studies. Kidney, liver, lung, stomach, and testes toxicity occurred at >90-times the MRHD. Toxicities included: kidney tubular degeneration, necrosis/apoptosis, and increased plasma and urine biomarkers; liver increased organ weight and cytoplasmic rarefaction (glycogen accumulation), centrilobular hypertrophy, and plasma ALT biomarker; stomach erosion and necrosis; testes gross changes (decreased size, prominent tubules) and germ cell depletion, mineralization, and epididymal dilatation; and, lung increased alveolar macrophages suggestive of phospholipidosis. Toxicity suggestive of phospholipidosis in lung was seen in short term rat studies and chronic lifetime treatment with >400-times the MRHD caused lung cholesterol cleft granuloma(ta). The clinical risk of phospholipidosis is considered minimal based on the large safety margin relative to the clinical dose.

Linagliptin was not genotoxic in a standard battery of *in vitro* and *in vivo* assays (Ames, *in vitro* CHO cell chromosome aberration in human lymphocytes, and *in vivo* repeat dose (4-week) rat micronucleus). Metabolite CD 1750 was also negative for mutagenicity (Ames assay) and clastogenicity (*in vitro* HPBL chromosome aberration assay).

The Sponsor also conducted genotoxicity studies for several drug substance impurities and additional 'potential' impurities identified in the drug product. Two impurities were positive for genotoxicity in *in vitro* assays but human exposure estimates are below levels that pose any carcinogenic risk. In addition, all of the drug substance impurities were present in all pivotal rat or monkey studies (usually both species) and in the

[4] DPP4 activity and structural homologue (DASH) family of serine proteases.

carcinogenicity studies. All listed impurities and potential or theoretical drug substance and drug product impurities were adequately qualified and levels are considered sufficiently low to pose insignificant toxicologic risk.

Carcinogenicity was assessed in chronic, lifetime oral gavage studies in mice and rats at doses that provided several hundred-fold higher exposure than experienced clinically. Linagliptin caused drug-induced lymphomas in female mice at 287-times the MRHD. No other drug-related tumors were seen in mice or rats. The NOAEL for drug-related tumors in female mice provided a 34-fold margin over expected human exposures. Linagliptin poses minimal carcinogenic risk to humans based on high exposure multiples at the NOAEL for drug-related tumors (34X) and very high exposure multiples (287X) at the tumorigenic dose in female mice. In addition, no drug-related tumors were found in rats exposed to over 400-times the MRHD. The very high exposure multiples achieved in rodents reflect the limited toxicity of linagliptin and a lowering of the clinical dose as drug development progressed from earlier clinical trials.

Reproductive and developmental toxicity were assessed in fertility, early embryonic development, and pre- and post-natal development studies. Linagliptin was not teratogenic at up to 30 mg/kg in rat (49X MRHD) and 150 mg/kg in rabbit (1943X MRHD). Very high, maternally toxic doses in rats (1000X MRHD) resulted in slightly decreased number of corpora lutea and embryofetal survival, increased late resorptions, and a slight 3-4% increase in rib malformations (flattened and thickened) above the historical range. There were no treatment-related fetal malformations in rabbits. Rabbit fetal variations of small gall bladder/hypoplasia and increased lumbar ribs (summa) were increased at high doses (>1000-times MRHD) compared to concurrent and historical controls. Treatment with 240 mg/kg (>800X MRHD) in male and female rats prior to mating did not have any apparent effect on fertility. Rats dosed during pregnancy ($F_0$) and throughout lactation ($F_1$) with 300 mg/kg linagliptin (> 1000X MRHD) resulted in offspring with lower birth weight that persisted to adulthood, delays in several physical and learning/memory developmental endpoints, and a reduced number of viable offspring ($F_2$) after mating. No functional, behavioral, or reproductive toxicity was observed in offspring of rats exposed to 49 times the clinical dose.

Summary of nonclinical issues relevant to clinical use:

1. Hypersensitivity / pseudoallergy may occur in susceptible individuals in the clinical population based on the findings in dogs and minipigs. The evidence suggests that this reflects a histamine-related response rather than an immunoglobulin-mediated allergic response. This reaction is distinct from the ulcerative necrotic skin lesions associated with some members of the DPP4 inhibitor class. Linagliptin did not cause necrotic skin lesions in any species in the non-clinical program.

2. The overall non-clinical toxicity profile suggests minimal target organ risks in humans. However, since DPP4 cleaves substrates other than the targeted incretin hormones, inhibition of DPP4 may have unintended consequences with

prolonged dosing that were not evident in the nonclinical program. As noted in the Januvia review “Effects on human immunity, specifically recall responses to antigens and immune cell trafficking, may be adversely affected by DPP4 inhibition. This risk is an unavoidable characteristic of…the drug class.”

3. Pancreatitis has arisen as a safety concern for GLP1 targeted therapeutics, including the DPP4 inhibitors. Linagliptin did not cause histological changes in the pancreas of animals indicative of pancreatitis or pancreatic injury, despite long-term exposure to very high doses of drug. A limitation to extrapolating these studies to the intended diabetic clinical population is that toxicity studies are conducted in normoglycemic healthy animals.

4. Linagliptin readily crosses the placenta and is secreted in milk in rats at approximately 4-times higher concentrations than maternal plasma. Fetal exposure was confirmed in rats and rabbits and assumed in nursing rats based on good overall oral bioavailability and the absence of evidence that linagliptin would be retained in milk and not absorbed in nursing pups. No specific risks from reproductive toxicity studies are predicted for fetuses, neonates, or nursing infants at clinical exposures, nevertheless, animal data support a conclusion that human fetuses and nursing infants will be exposed to linagliptin from maternal drug use.

## 1.3 Recommendations

### 1.3.1 Approvability

Nonclinical data support the safe use of linagliptin under the proposed uses. The pharmacology/toxicology reviewer recommends approval.

### 1.3.2 Additional Non Clinical Recommendations

No additional studies are needed.

### 1.3.3 Labeling

<u>8.1 Pregnancy</u>
*Pregnancy Category B is acceptable*

Paragraph #1 [redacted] (b) (4) –
“Reproduction studies have been performed in rats and rabbits. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.”

Paragraph #2 (Edit) – “Linagliptin administered during the period of organogenesis was not teratogenic at doses up to 30 mg/kg in the rat and 150 mg/kg in the rabbit, or approximately 49- and 1943-times the clinical dose based on AUC exposure. Doses of linagliptin causing maternal toxicity in the rat and the rabbit also caused development delays in skeletal ossification and slightly increased embryofetal loss in rat (1000 times the clinical dose) and increased fetal resorptions and visceral and skeletal variations in the rabbit (1943 times the clinical dose).”

Paragraphs #3 and #4 (Add) – “Linagliptin administered to female rats from gestation day 6 to lactation day 21 resulted in decreased body weight and delays in physical and behavioral development in male and female offspring at maternally toxic doses (exposures >1000 times the clinical dose). No functional, behavioral, or reproductive toxicity was observed in offspring of rats exposed to 49 times the clinical dose.

Linagliptin crossed the placenta into the fetus following oral dosing in pregnant rats and rabbits.”

<u>8.3 Nursing Mothers</u>
Sentence #1 (edit) – “Available animal data have shown linagliptin is excreted in milk *at a milk to plasma ratio of 4:1*.”

<u>12.2 Pharmacodynamics</u>
Sentence #3 (change) – “Linagliptin binds selectively to DPP-4 and *selectively inhibits DPP-4 but not* DPP-8 or DPP-9 *in vitro at concentrations approximating therapeutic exposures.*”

<u>13.1 Carcinogenesis, mutagenesis, impairment of fertility</u>
Final Sentence, Paragraph #1 (edit) – (b) (4) [redacted]

# 2 Drug Information

## 2.1 Drug

Tradjenta® (proposed)

### 2.1.1 CAS Registry Number

668270-12-0

### 2.1.2 Generic Name

Linagliptin

### 2.1.3 Code Name

BI 1356 BS

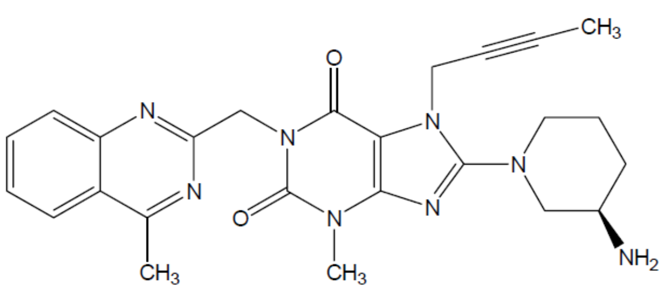
### 2.1.4 Chemical Name

1H-purine-2,6-dione, 8-[(3R)-3-amino-1-piperidinyl]-7-(2-butynyl)-3,7-dihydro-3-methyl-1-[(4-methyl-2-quinazolinyl)methyl]-

### 2.1.5 Molecular Formula/Molecular Weight

$C_{25}H_{28}N_8O_2$ / 472.54 g/mol

### 2.1.6 Structure (or Biochemical Description)



Linagliptin has one chiral center. The R-enantiomer, termed BI 1356 BS, is the proposed drug substance. Neither interconversion to the S-enantiomer racemate, BI 1355 BS, nor degradation during storage, showed meaningful amounts of S-enantiomer in the drug substance [redacted] (b) (4) R-enantiomer).

### 2.1.7 Pharmacologic class

Dipeptidyl peptidase IV (DPP4) inhibitor.

## 2.2 Relevant IND/s, NDA/s, and DMF/s

Linagliptin INDs – IND 70,963 (linagliptin); IND 105,055 (linagliptin + metformin FDC); IND 108,288 (linagliptin + pioglitazone); IND 108,388 (linagliptin + BI10773)

DPP4 Inhibitor NDAs – NDA 21-995 (sitagliptin, Januvia®) and NDA 22-044 (sitagliptin + metformin, Janumet®); [redacted] (b) (4) (vildagliptin); NDA 22-350 (saxagliptin, Onglyza®) and NDA 200678 (saxagliptin + metformin, Kombiglyze XR ®); [redacted] (b) (4) (alogliptin) and [redacted] (b) (4) (alogliptin + pioglitazone)

## 2.3 Drug Formulation

The proposed drug product is a single dosage form, 5 mg linagliptin immediate release film-coated tablet. A summary of excipients in the drug product is shown in Table 1. All of the excipients [redacted] (b) (4) conform to USP or NF monographs. [redacted] (b) (4), conforms to a company standard and actual components are shown separately in the bottom of Table 1. All of the individual components of the [redacted] (b) (4) formulation conform to USP or NF. Different formulations [redacted] (b) (4) have been used in current or previously listed drug products but the current formulation has not been approved. All of the individual inactive ingredients [redacted] (b) (4) have been previously included at higher concentrations as inactive ingredients in oral drugs and printing inks are food-grade.

**Table 1 – Composition of drug product and excipients list**

Qualitative and quantitative composition of linagliptin film-coated tablets, 5 mg

| Part of tablet | Ingredient | [mg/coated tablet] | Function | Reference to Standards |
|---|---|---|---|---|
| Tablet core | Linagliptin | 5.000 | Active | Company Standard |
| | Mannitol | [redacted] (b) (4) | | USP |
| | Pregelatinized Starch | | | NF |
| | Corn Starch | | | NF |
| | Copovidone | | | NF |
| | Magnesium stearate | | | NF |
| | [redacted] (b) (4) | | | |
| Coating | | | | |
| | **Total weight** | **185.000** | | |

* Removed during processing, does not appear in the final product

[redacted] (b) (4)

The nature of the drug substance formulation results in a long list of actual and theoretical impurities. Table 2 shows acceptance criteria for organic impurities, organic volatile impurities, and residual solvents. Table 3 show solvent classification and limits from ICH Q3C guidance for residual solvent concentrations in drug substances[5]. Maximum solvent concentrations from drug substance batches were all below ICH limits (Table 3). The listed impurities required qualification based on [redacted] (b) (4) in the drug substance based on 5 mg daily dose[6]. All of the listed organic impurities were qualified for toxicity individually in genetic toxicity assays and *in vivo* in nonclinical toxicology studies (see nonclinical batch analyses in Table 4, below). Drug substance impurities

[5] ICH Q3C Guidance for Industry, Residual Solvents, 1997 and Tables and Lists, 2003
[6] ICH Q3A(R2) Guidance for Industry Impurities in New Drug Substance, 2008

were also present in batches used for two year mouse and rat carcinogenicity studies, which showed no evidence of drug-related tumors up to 34- and 418-times the clinical dose in mice and rats, respectively.

Impurities that were found to be genotoxic or otherwise posed a theoretical concern are discussed in detail in Section 2.5, below.

**Table 2 – Drug substance specification and impurity summary**

(b) (4)

[redacted]

**Table 3 – Residual solvent limits and observations in drug substance**

(b) (4)

[redacted]

## 2.4 Comments on Novel Excipients

None. All excipients are compendial or have been previously approved in listed drug products.

## 2.5 Comments on Impurities/Degradants of Concern

The FDA chemistry (CMC) review contains a comprehensive list of all actual and potential impurities and degradants in the drug substance and drug product[7]. The Sponsor and CMC reviewer identified several impurities and/or degradants with potential toxicologic concern. Substances discussed here are limited to those that were genotoxic or identified for toxicity analysis by the CMC reviewer. All other impurities were not genotoxic *in vitro* in Ames mutagenesis and chromosome aberration assays for clastogenic potential (see Section 7, below). Batch analyses of impurities and estimated margins to clinical exposure are summarized in Table 4. Exposure margins in Table 4 are based on NOAELs from chronic rat or monkey studies, which show >96-fold excess over therapeutic exposures. Overall, all impurities and degradants were qualified in accordance with current guidance and none are considered to pose a significant toxicologic risk.

(b) (4)

[redacted]

[7] NDA 201280 CMC review (S. Markofsky, 3/7/11)

(b) (4)

[redacted]

[8] McGovern T and Jacobson-Kram D. 2006. Trends Anal Chem 25:790-795.
[9] *IBID*
[10] ICH Guidance for Industry Q3B(R2) Impurities in New Drug Products, 2006

(b) (4)

[redacted]

## 2.6 Proposed Clinical Population and Dosing Regimen

Indicated for treatment of Type 2 diabetes mellitus (T2DM). A single proposed oral tablet dose of 5 mg QD is proposed.

## 2.7 Regulatory Background

NDA 201280 is the original submission of linagliptin as a new molecular entity under 505(b)(1). [redacted] (b) (4)

# 3 Studies Submitted

## 3.1 Studies Reviewed

Studies reviewed in the NDA are listed in the Table of Contents. Most studies were previously submitted and reviewed during the IND phase for linagliptin (IND 70,963).

Summaries of nonclinical study reviews from IND 70,963 are included throughout this NDA review.

### 3.2 Studies Not Reviewed

None.

### 3.3 Previous Reviews Referenced

None.

# 4 Pharmacology

### 4.1 Primary Pharmacology

Linagliptin is indicated for treatment of type 2 diabetes mellitus as an adjunct to diet and exercise. It is an orally active dipeptidyl peptidase IV (DPP4) inhibitor. DPP4 is a serine protease that cleaves polypeptides at [redacted] (b) (4) residues at the N-terminus. DPP4 is typically expressed on the cell surface of a wide variety of tissues, including kidney, liver, intestine, lymphocytes, and vascular endothelial cells. DPP4 is also present and active in plasma, which is the therapeutic target and thought to be derived from shedding of intact, active DPP4 from cell surfaces. DPP4-mediated cleavage regulates a variety of peptides including peptide hormones, neuropeptides, and chemokines. Regulation of incretin hormones in the gut, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), seems to be a critical physiologic role of DPP4. The incretins, in combination but with GLP-1 playing a key role, increase insulin secretion, enhance insulin receptor sensitivity, and decrease hepatic glucose, gastric emptying, and food intake. Inhibition of DPP4 prevents the natural rapid breakdown of GLP-1 and GIP after their postprandial expression.

DPP4 inhibition has been shown to reduce blood sugar and glycated hemoglobin ($HbA1_c$) *in vivo* in healthy and diabetic animal models and in diabetic patients. Two DPP4 inhibitors, sitagliptin (Januvia™) and saxagliptin (Onglyza®) are currently marketed in the U.S. and globally for treatment of type 2 diabetes. A third DPP4 inhibitor, vildagliptin, is currently marketed in various countries outside the U.S. In addition, exenatide, an incretin mimetic, and liraglutide, a synthetic peptide, are also marketed in the U.S. Exenatide and liraglutide are mimetics/analogs of glucagon-like peptide 1 (GLP-1), which are cleaved and inactivated by DPP4 and thus the intended pharmacodynamic effect of increased GLP-1 activity is similar for GLP-1 mimetics and DPP4 inhibitors. Linagliptin is currently proposed as a monotherapy as an adjunct to diet and exercise and as add-on therapy to other oral diabetic therapies (metformin, sulfonylurea, or thiazolidinedione) when glycemic control becomes inadequate.

Linagliptin is a high potency, competitive, reversible inhibitor of DPP4. High potency was shown *in vitro* in human CaCo-2 cell extracts, with linagliptin $IC_{50}$ = 1 nM. Similar potency and activity were shown in human plasma, with linagliptin $IC_{50}$ = 3.6 nM. Linagliptin inhibits DPP4 to a similar degree in multiple animal species, with $IC_{50}$s of 6.8, 10.2, and 10.4 nM in rat, mouse, and dog, respectively (see Table 5). Linagliptin has a high affinity for direct DPP4 protein binding. The high affinity DPP4-binding affects linagliptin pharmacokinetics and influences concentration-dependent DPP4 inhibition characteristics *in vivo*. Modeling and binding studies showed a DPP4 binding affinity constant of 231 pM and saturation of binding at approximately 1 nM linagliptin. DPP4 was measured in plasma at approximately 5 nM concentrations. Thus, protein binding saturation occurs at the same order of magnitude as inhibition. While protein binding clearly affects pharmacokinetics, oral dosing with linagliptin readily exceeds saturating plasma concentrations and achieves potent, durable plasma DPP4 inhibition *in vivo*. DPP4-binding characteristics were investigated in detail and are discussed more thoroughly below.

**Table 5 – Sponsor's DPP4 inhibition species comparison**

DPP-4 inhibition by linagliptin (plasma of various species)

| Plasma source | $IC_{50}$ [nM] |
|---|---|
| Human | 3.6 |
| Mouse | 10.2 |
| Rat | 6.8 |
| Dog | 10.4 |

DPP4 inhibition by the major metabolite, CD 1790, was also investigated *in vitro*. CD 1790 showed no DPP4 inhibition at 1 µM and a maximum 61% DPP4 inhibition at 10 µM. DPP4 inhibition below 70-80% inhibition is considered not biologically significant. Assuming even minimal DPP4 inhibition *in vivo* at high concentrations of CD 1790, linagliptin is >1000-times more potent and CD 1790 is not expected to contribute to efficacy *in vivo*.

Linagliptin showed high selectivity for DPP4 inhibition (see Table 6). DPP4 has several highly related proteins, together making up the DASH (DPP4 activity and structural homologue) family of proteins. Fibroblast activated protein (FAP) is the most highly homologous to DPP4 and it is the only enzyme which had appreciable linagliptin-mediated inhibition ($IC_{50}$ = 94 nM). Potential for off target effects on hemolysis or wound healing due to FAP inhibition were investigated in several dedicated studies and linagliptin treatment had no significant *in vivo* effects. Linagliptin had no significant modulating effect on an extensive panel of receptors, ion channels, and enzymes screened *in vitro*.

Linagliptin showed > 10,000-fold selectivity for DPP4 inhibition compared to the related dipeptidyl peptidases DPP8 and DPP9 (Table 6). DPP8 and DPP9 are also closely related cytoplasmic enzymes differentially expressed in skeletal muscle, heart, liver, and activated T-cells. While the physiological role of DPP8 and DPP9 are still unknown, inhibition of DPP8/9 have been associated with animal toxicity including alopecia, histopathological changes in multiples organs, gastrointestinal toxicity, and blood and immune system effects (thrombocytopenia, reticulocytopenia, enlarged spleen) which may be mediated by inhibition of T-cell proliferation (Lankas GR et al., Diabetes (2005) 54(10):2988). Skin, immune, and GI-related toxicity have been observed with some DPP4 inhibitors and, based on the similar toxicity profile with selective DPP8 and/or DPP9 inhibition, some toxicity attributed to DPP4 inhibition may be due to off-target inhibition of DPP8/9.

**Table 6 – Sponsor's DPP4 inhibition selectivity**

Inhibition of DPP-4 related proteases by linagliptin

| Enzyme | Inhibition |
|---|---|
| DPP-4 | $IC_{50}$ = 1 nM |
| FAP | $IC_{50}$ = 94 nM |
| DPP-2 | No inhibition at 100 μM |
| DPP-8 | $IC_{50}$ = 40 μM |
| DPP-9 | $IC_{50}$ = 11 μM |
| POP/PEP | No inhibition at 100 μM |
| APN | $IC_{50}$ >100 μM |
| APP | No inhibition at 10 μM |
| Plasmin | No inhibition at 100 μM |
| Trypsin | No inhibition at 100 μM |
| Thrombin | No inhibition at 100 μM |

Linagliptin pharmacology was assessed in a variety of *in vitro* and *in vivo* animal models to investigate DPP4 inhibitory activity and effects on blood glucose. Pharmacology trends are discussed and summarized below.

Blood glucose lowering effects are expected at approximately 80% DPP4 inhibition. *In vivo* DPP4 inhibition occurred rapidly, within 30 min after oral linagliptin dosing in healthy rat, dog, and monkey models. Effective DPP4 inhibition was shown for prolonged periods ≥ 7 h after single oral linagliptin doses in rat (0.26 mg/kg), dog (1 mg/kg), and rhesus monkey (1 mg/kg). Slightly higher doses resulted in DPP4 inhibition for 24 h (e.g., 0.69 mg/kg in rat). Similar *in vivo* DPP4 inhibition was seen in diabetic animal models, for example ≥ 70% inhibition in Zucker Fatty (*fa/fa*) rats within 20 min after 1 mg/kg linagliptin by oral gavage.

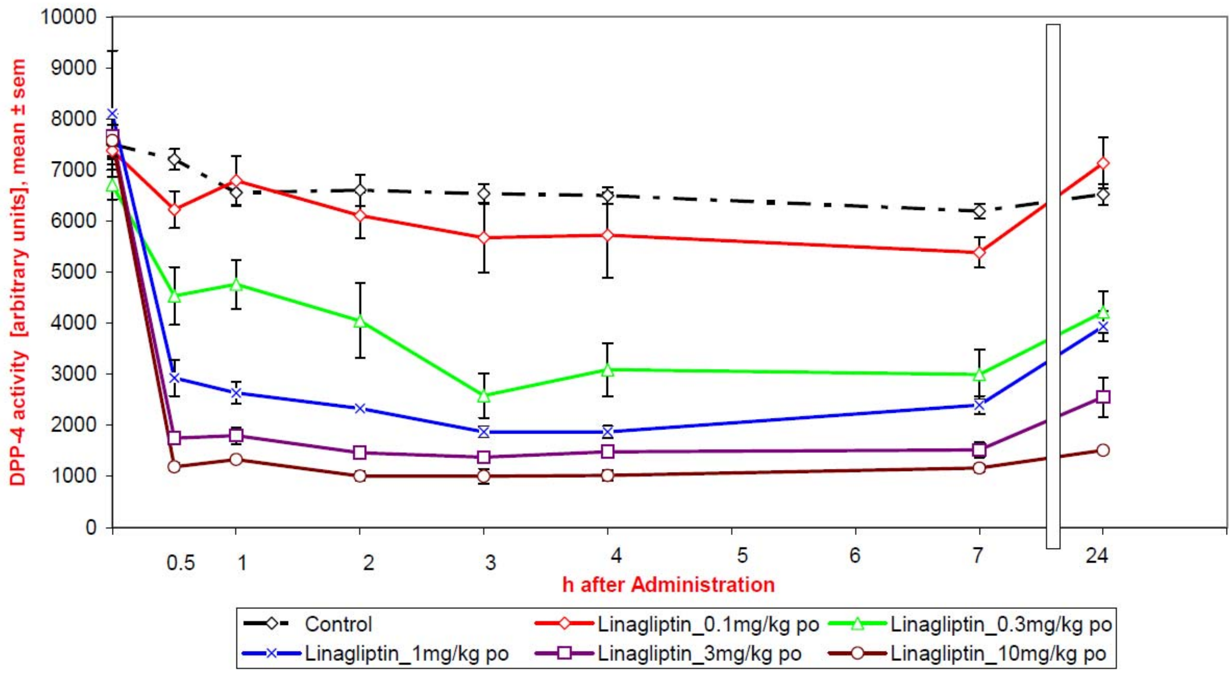
Glucose lowering effects of linagliptin treatment were also demonstrated *in vivo*. Results from various animals, including diabetic models, are shown in the Sponsor's summary table (Table 7). Figure 1 shows dose-related inhibition of plasma DPP4 from healthy rats treated with a single oral gavage dose of linagliptin. Results also show the durable effect of linagliptin treatment, with rapid DPP4 inhibition by 0.5 h post-dose and sustained inhibition to 7 h post-dose and continuing to 24 h post-dose in efficacious doses. Studies in dog and rhesus monkey showed DPP4 inhibition after a single 1 mg/kg oral dose lasted 48 h (dog) to 72 h (monkey).

**Table 7 – Sponsor's *in vivo* pharmacology summary**

Effect of linagliptin on glucose homeostasis in various animal models

| Animal model | Dosage, Duration (all by oral gavage) | Test, Parameter | Effect |
|---|---|---|---|
| C57BL/6J mouse | 0.3 mg/kg linagliptin<br>Single dose | oGTT,<br>Peak glucose (30 min) | -32.9%<br>AUC glucose |
| *db/db* mouse | 0.1, 0.3 & 1 mg/kg linagliptin,<br>Single dose | oGTT,<br>$ED_{50}$, AUC glucose | $ED_{50}$ < 1mg/kg |
| *db/db* mouse | 3 mg/kg linagliptin<br>200 mg/kg metformin<br>Single dose | oGTT,<br>AUC glucose | -37%<br>AUC glucose |
| *db/db* mouse | 3 mg/kg linagliptin<br>8-week repeat-dose | HbA1c | ~1% HbA1c |
| *fa/fa* rat | 1 mg/kg linagliptin<br>Single dose | oGTT,<br>AUC glucose | -38.5%<br>AUC glucose |
| ZDF rat | 1 mg/kg linagliptin<br>Single dose | oGTT,<br>AUC glucose | -19.5%<br>AUC glucose |

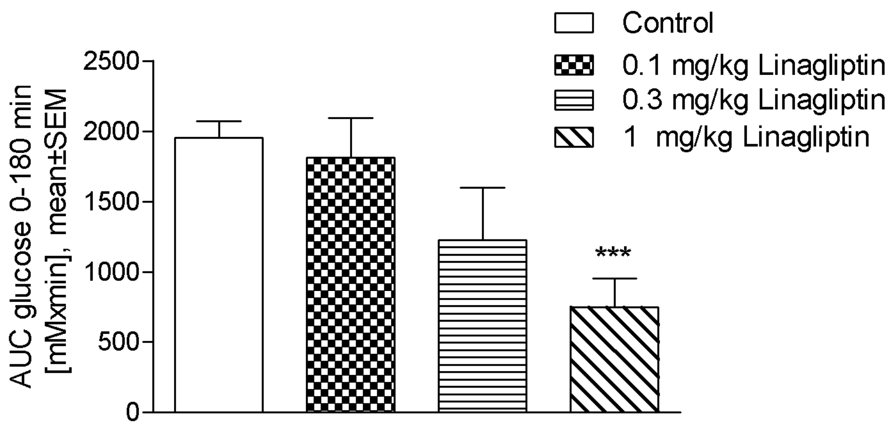
**Figure 1 – *In vivo* DPP4 inhibition in rat**




Dose dependent effect of linagliptin on plasma DPP-4 activity in Wistar rats after oral administration [U05-1995]

Figure 2 shows dose-related improvement of glucose excursion in an oral glucose tolerance test (OGTT) in diabetic *db/db* mice. Total glucose excursion was suppressed by 62% in mice treated with 1 mg/kg linagliptin, demonstrating an improved response to glucose load in the model of insulin resistance and glucose intolerance.

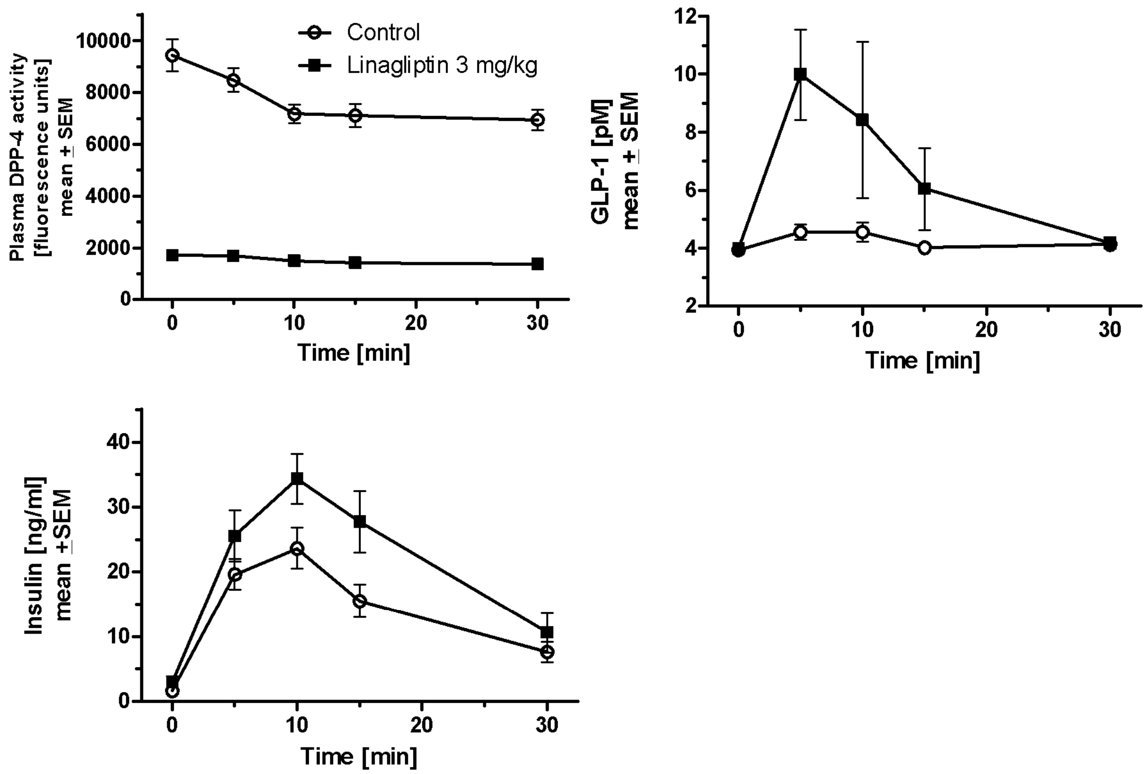
**Figure 2 – Improved glucose excursion in *db/db* diabetic mice**




Effect of oral administration of linagliptin (0.1, 0.3 or 1 mg/kg) on AUC following an oral glucose tolerance test in *db/db* mice. Indicated p-values above bars are calculated versus control (***, p< 0.001). [U05-1995]

Linagliptin treatment in Zucker fatty rats (*fa/fa*), a model of diet-induced glucose intolerance, confirmed rapid and durable DPP4 inhibition after oral dosing. DPP4 activity was inhibited >70% by 20 min post-dose, leading to improved glucose excursion in OGTT, with ≥ 1 mg/kg linagliptin. Durability of DPP4 inhibition and GLP-1 and insulin response were also shown to be improved by 3 mg/kg linagliptin treatment. DPP4 remained inhibited >16 h and glucose-dependent GLP-1 expression and insulin response were increased (Figure 3).

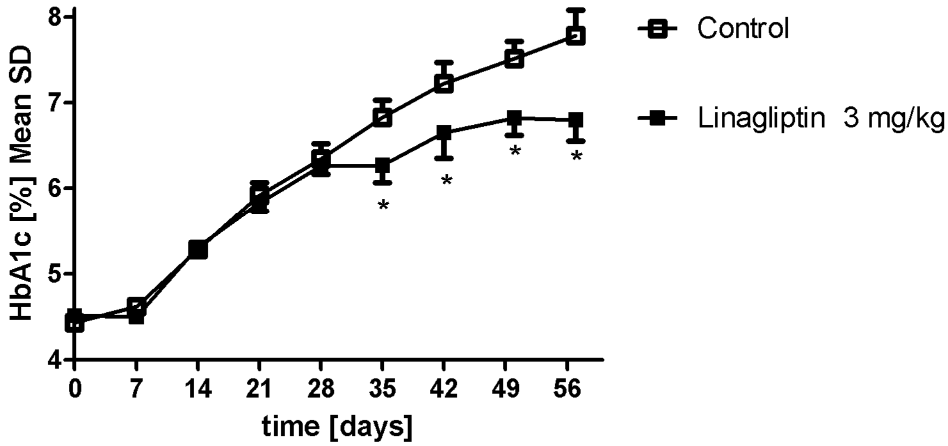
**Figure 3 – DPP4 inhibition, GLP-1, and insulin in *fa/fa* rat**




Effect of oral administration of linagliptin (16 h prior oGTT) on DPP-4 activity, glucose-induced elevations in plasma GLP-1 and insulin levels in *fa/fa* rats [U05-1995, Module 4.2.1.1]

Long term efficacy was assessed in diabetic *db/db* mice treated with 3 mg/kg linagliptin by oral gavage for 8 weeks. Treatment commenced in 8-week old mice and $HbA_{1c}$ and insulin sensitivity (by euglycemic insulin clamp) were assessed after the 8-week treatment. $HbA_{1c}$ levels increase over time in the diabetic animals and linagliptin significantly reduced $HbA_{1c}$ compared to controls. Plasma $HbA_{1c}$ leveled off after approximately 6 weeks of treatment, while levels continued to increase throughout the study in controls (Figure 4). Effects of long term treatment in this model were only modest for improvement of insulin sensitivity (nss) and improvement of fed plasma glucose (ss) and there was no improvement in glucose tolerance at the end of treatment. Insulin resistance and plasma glucose control continued to deteriorate over the course of the study, which is consistent with the severe insulin resistance in the model. In a separate study in *db/db* mice, one-week treatment with either 1 mg/kg linagliptin or 200 mg/kg metformin only slightly improved glucose tolerance (13% and 19% decreased glucose excursion, respectively; nss). Combination linagliptin plus metformin showed an additive effect on oral glucose tolerance, with a significant 37% reduction in glucose excursion after one week treatment.

**Figure 4 – $HbA_{1c}$ in *db/db* mice treated with linagliptin**




Linagliptin and other DPP4 inhibitors are not expected to have any effect in the absence of a glucose load because the target incretin hormone GLP-1 is released after meals. In support of that hypothesis, similar to other DPP4 inhibitors linagliptin treatment did not increase plasma insulin or reduce plasma glucose in non-diabetic animals. Risks of hypoglycemic events due to DPP4 inhibition are expected to be lower than some diabetic therapies such as sulfonylureas or insulin, which do lower plasma glucose in fasted rats.

## 4.2 Secondary Pharmacology

Neither linagliptin nor the major metabolite CD 1790 were implicated for effects on pharmacology unrelated to DPP4 inhibition.

CD 1750 (racemic mixture, predominantly the CD 1790 R-enantiomer) showed maximum 61% DPP4 inhibition at 10 μM *in vitro*, which is not considered to be pharmacological significant (i.e., ≥ 70-80 inhibition). Compared to parent, CD 1750 was >1000-fold less potent for DPP4 inhibition. CD1790 was also screened *in vitro* for potential off-target effects on various receptors and enzymes. The only notable effect on receptor binding or enzyme inhibition and/or induction were moderate, 51% induction of $Cox_1$ and $Cox_2$ at 10 μM. The Sponsor did not consider Cox enzyme induction biologically meaningful, noting apparent induction at high drug concentrations “are generally attributable to non-specific effects of the test compounds in the assay”. Regardless, potential Cox induction occurred at concentrations approximately 10,000-fold higher than linagliptin’s expected therapeutic effect.

Potential for linagliptin and a reference DPP4 inhibitor to aid in wound healing were investigated in male C57bl6 mice given surgical wounds on their back. Oral doses up to 30 mg/kg linagliptin for 14 days had no effect on wound healing. A reference DPP4 inhibitor (not identified) showed a similar absence of wound healing.

P-glycoprotein (P-gp) mediated transport of linagliptin was observed in CaCo-2 cells. Transport was concentration-dependent and a very high $K_m$, >100 μM, was estimated for P-gp. Linagliptin was considered to be a “moderately permeable” compound and also showed low potency inhibition of P-gp ($IC_{50}$ ≈ 55 μM). The potential *in vivo* role of P-gp on linagliptin membrane transport was investigated in rats. Intestinal transport and oral bioavailability were assessed in the presence and absence of a potent, selective P-gp inhibitor (Zosuquidar). Pre-treatment with Zosuquidar caused a marked increase in linagliptin plasma $C_{max}$ and AUC, indicating increased intestinal absorption and bioavailability in the presence of P-gp inhibition. Results suggest potential drug-drug interactions when linagliptin is taken concomitantly with drugs that inhibit P-gp. Therapeutic doses of linagliptin are not expected to cause direct P-gp inhibition due to the high potency and selectivity of linagliptin for DPP4 inhibition.

Linagliptin transport and inhibition trends with a variety of other SLC (OAT and OCT transporters) and ABC (MDR1, MRP2, BCRP) drug transporters *in vitro*. Linagliptin showed limited potential for inhibition of OAT and OCT transporters, with maximum inhibition of OCT1 and OATP2 at $IC_{50}$s of 45 μM and 70 μM, respectively. Linagliptin cellular efflux was increased only by MDR1, showing linagliptin was a MDR1 substrate with an approximate $K_m$ of 187 μM. Linagliptin also inhibited MDR1-mediated transport, with an $IC_{50}$ of 66 μM. Based on the high potency of linagliptin for DPP4 inhibition, there is minimal potential for linagliptin-mediated inhibition of drug transporters at clinically relevant concentrations.

## 4.3 Safety Pharmacology

A variety of dedicated safety pharmacology studies were conducted with linagliptin. Individual analyses and overall conclusions from safety pharmacology studies are shown below.

> **“In vitro hERG patch clamp evaluation in HEK-293 cells:** BI 1356 BS was tested for inhibition of the cloned hERG potassium channels expressed in HEK-293 cells by using whole cell voltage-clamp methods. At the concentrations of 1, 3, and 10μM, BI 1356 BS had no effect on HERG currents thereby excluding the calculation of an IC50 value.
>
> **In vitro isolated guinea pig papillary muscle action potential study:** BI 1356 BS was tested for the effects on cardiac action potentials in guinea pig papillary muscle. At the concentrations up to 10μM, BI 1356 BS had no effect on resting membrane potential, action potential, amplitude and overshoot and maximal upstroke velocity. However, there was a concentration-dependent shortening of the action potential beginning at 0.3μM and increased up to a 7% shortening (of APD90) at 10μM; APD30 and APD10 were affected similarly. The force of contraction was also increased in a concentration-dependent manner beginning within 0.3μM.

**Cardiovascular safety pharmacology:** Single dose cardiovascular safety pharmacology study of BI 1356 BS was tested in both rats and dogs. In the rat study, BI 1356 BS was tested at doses of 3, 10, or 30mg/kg in previous instrumented rats for the telemetric transmission of signals for arterial blood pressure, ECG (not used for determination of QT interval) and body temperature. The measurement were made up to 7 hours post-application, and BI 1356 BS had no effect on any of the parameters measured at the tested doses. In the conscious dog study, BI 1356 BS was tested at doses of 1, 3 or 10mg/kg and the hemodynamic data were collected continuously over the subsequent 7 hours. In addition, a single blood sample was taken at the end of study which confirmed high drug exposure. There were no effects on arterial blood pressure, left ventricular pressure, ECG and temperature following the administration of BI 1356 BS. Highest doses tested in rats and dogs are approximately 1X the MRHD of 400mg based on body surface area.

**Neuro-functional safety pharmacology:** Possible effects of BI 1356 BS on the central and peripheral nervous systems were investigated in mice and rats. In the mice, a modified Irwin test (behavioral assessment) and assessing the spontaneous nocturnal motility were used. In the male rat study, effects of BI 1356 BS on behavior, physiological state, spontaneous locomotor activity or body temperature were evaluated. No effect of BI 1356 BS was observed in mice at doses up to 30mg/kg and in rats at doses up to 600mg/kg.

**Respiratory safety pharmacology:** BI 1356 BS was tested at doses of 6, 60 or 600mg/kg in male rats. There was no effect on respiratory rate, tidal volume, and minute volume at doses of 6 and 60mg/kg. There was an isolated decrease in respiratory rate and subsequently minute volume without an effect on tidal volume 30 minutes after administration of 600mg/kg BI 1356 BS.

**Gastrointestinal system safety pharmacology:** BI 1356 BS was tested on the gastrointestinal system in rat at doses of 3, 10 or 30mg/kg given either orally or intraduodenally. After intraduodenal administration of BI 1356 BS, the effect on gastric section was assessed in pylorus-ligated rats. BI 1356 BS at doses of 10 and 30mg/kg led to a slight reduction in gastric juice volume and thereby acid output. There was no effect on the rate of gastric emptying following the oral administration. However, there was a slight dose-dependent reduction in the intestinal transit after each oral dose.

**Renal and clinical chemistry safety pharmacology:** BI 1356 BS was tested for effects on renal function and clinical chemistry parameters in rats at doses of 3, 10, or 30mg/kg. BI 1356 BS in the tested doses had no effect on renal function, urinalysis, and serum electrolytes. At dose of 30mg/kg, BI 1356 BS increased plasma protein, cholesterol, free fatty acids, total bilirubin and ALT.

**Safety pharmacology conclusions:** In the in vitro safety pharmacology studies, a concentration-dependent shortening of the action potential beginning at

0.3μmol/l and increased up to a 7% shortening at 10μmol/l in the action potential of the Guinea pig papillary muscle. However, there are no changes of blood pressure, heart rate in rats and dogs, and no changes of EKG at doses up to 30mg/kg in rats and 10mg/kg in dogs. Other drug-related findings in the safety pharmacology studies included a decreased respiratory rate and minute volume at 600mg/kg, increased protein, cholesterol and free fatty acids at 30mg/kg, and a slight reduction in gastric juice volume and acid output at doses of 10 and 30mg/kg in rats."

# 5 Pharmacokinetics/ADME/Toxicokinetics

## 5.1 PK/ADME

**Analytical Methods and Validation**

Bioanalytical methods were validated for extraction, quantification, and stability of linagliptin and metabolite CD 1750 XX in plasma of all experimental animal species used in pivotal pharmacology and toxicology studies. Some of the methods were originally partially validated and fully validated in further analyses. Methods described as “cross” validation described methods validated across multiple laboratories. The methods were rigorous and adequate to quantify both drugs from mouse, rat, rabbit, dog, minipig, and monkey plasma. Additional analytical methods were described and reviewed during the IND phase, including methods for linagliptin quantification in human plasma and human urine, CD 1790 XX in human plasma, and whole blood $HbA_{1c}$ levels. Study results and linear range for plasma analyses are briefly described below.

**Absorption**

Linagliptin is rapidly absorbed after oral administration, with maximum plasma concentrations ($T_{max}$) typically occurring by 30-60 min postdose in animal models and $T_{max}$ = 1.5 h in humans. Biphasic absorption was observed in rats after a single oral dose, with a rapid plasma $T_{max}$ at 30 min, followed by a second, lower, plasma peak 4 h post-dose. The biphasic absorption was not observed after multiple dose exposure. Oral bioavailability ranged from 18% to 69% across species and strains.

Plasma half lives ($t_{1/2}$) were also high in all species, ranging from a minimum $t_{1/2}$ of 10-12 h after high dose exposures in rabbit and rat and up to 131 h in humans at the clinical dose. The volume of distribution was also high in all species (>5 L/kg), consistent with extensive tissue distribution. Variability in $t_{1/2}$ between low and high doses was evident in different species, with shorter $t_{1/2}$ in higher dose treatments. The high tissue distribution and variable $t_{1/2}$ were attributable to saturable, high affinity binding to DPP4 in plasma and tissues. After saturation of plasma DPP4, which occurs at slightly higher concentrations than the calculated plasma $IC_{50}$ values, linear PK trends occur, tissue distribution stabilizes as tissue DPP4 is saturated, and linagliptin is more readily eliminated resulting in shorter $t_{1/2}$ compared to non-saturating conditions. DPP4 binding and distribution trends are discussed in detail below.

A comparison of pharmacokinetic parameters is shown in Table 8.

**Table 8 – Sponsor's oral PK summary across species**

Species comparison of mean pharmacokinetic parameters of the parent drug after single oral administration of linagliptin

| | Species | Mouse (CD-1) | | Rat (Wistar) | | Rabbit (Himalayan) | | Cynomolgus monkey | | Man |
|---|---|---|---|---|---|---|---|---|---|---|
| Para-meter | Gender: Unit | males | | males | | females | | males | males & females | males & females |
| **Dose** | mg/kg | 5 | 15 | 5 | 45 | 4 | 25 | 3 | 5 | 5mg/ subject |
| **$C_{max}$** | nM | 84.3 | 1010 | 547 | 6830 | 368 | 8560 | 622 | 1150 | 11.1 |
| **$t_{max}$** c) | h | 1.00 | 1.00 | 2.25 | 1.25 | 0.67 | 0.50 | 0.5-2 | | 1.53 |
| **$AUC_{0\text{-}inf}$** | nM· h | 422 | 3050 | 2610 | 4560 | 1330 a) | 16300 a) | 3740 | 4550 | 158 d) |
| **MRT** | h | 11.0 | 4.99 | 14.3 | 5.51 | 33.9 | 9.98 | 28.9 | 17.4 | 131 |
| **$t_{1/2}$** | h | 23.6 | 18.7 | 35.9 | 12.2 | 41.3 | 59.0 | 46.7 | 41.4 | 131 |
| **F** | % | 18.4 b) | 44.4 b) | 54.8 | | NC e) | NC e) | 68.8 | 41 | 30 f) |

NC = not calculated , arithmetic mean for animal species, geometric man for human data
a) AUC(0-96h) b) calculated across studies and different strains c) median values
d) AUC(0-24h) in steady state e) only oral studies performed f) across studies using model based approach

## Distribution

An extensive battery of studies investigated linagliptin distribution *in vitro* and *in vivo* after oral and *iv* dosing. Linagliptin binds to plasma protein in a concentration-dependent manner. Plasma protein binding was very high, >99%, at low linagliptin concentrations (below 1 nM) and moderately high, approximately 70% to 96%, at linagliptin concentrations above 1 nM (Table 9). There were slight differences in absolute protein binding across species, but trends were consistent with a saturable, high affinity binding to DPP4 followed by binding non-specifically to other plasma proteins. Because linagliptin has higher binding affinity to DPP4 than other plasma proteins, saturating concentrations of linagliptin lead to an increase in free drug in plasma. Plasma from humans dosed orally or *iv* was assessed for covalent (non-extractable) linagliptin binding, which showed minimal covalent binding (0.1%) at $T_{max}$ after oral dosing, with covalent binding increased to maximum 14% at 24h – 72h time points. Increasing non-extractable protein binding with increasing concentration was also consistent with other trends suggesting high affinity, saturable binding of linagliptin to DPP4.

**Table 9 – Sponsor's plasma protein binding summary**

Summary of *in vitro* plasma protein binding of linagliptin in various species

| Species | Strain | Concentration range tested [nM] | fB of linagliptin [%] |
|---|---|---|---|
| Mouse | CD-1 | 30-3000 | 78.2-72.2 |
| | C57BL/6J | 0.172 – 13100 | 99.3-74.5 |
| Rat | Crl:WI(Han) | 3-30 | 96.1-76.8 |
| | Fischer F344 | 3-30 | 95-4-80.7 |
| | Fischer F344 | 0.139 - 12700 | 99.2-77.5 |
| Rabbit (females) | Crl:CHBB(HM) | 30-3000 | 79.6-84.3 |
| Cynomolgus monkey | | 30-3000 | 82.0-70.4 |
| Human | | 0.021 – 29900 | 99.3-77.3 |

The volume of distribution was high in all species (>5 L/kg), consistent with extensive tissue distribution. Tissue distribution was confirmed in autoradiography studies. Initial studies showed very long plasma $t_{1/2}$ and tissue $t_{1/2}$ for selected tissues. The linagliptin tissue distribution pattern correlated well with patterns of DPP4 expression. Tissue linagliptin was highest in rat and mouse kidney, liver, lungs, spleen, and thymus, while DPP4 protein and mRNA expression were reported to be highest in kidney, lung, small intestine, liver, and spleen; low levels in other tissues, undetected in brain and muscle[11]. Investigations with DPP4-deficient rat and DPP4 knockout mouse models confirmed trends suggesting specific, saturable DPP4 binding in plasma and tissues. Study data are summarized in more detail below.

Autoradiography assessment in male albino rats showed saturable, drug-specific binding in kidney, liver, lungs, spleen, and thymus. Dose-related trends showed long residence times of linagliptin in the aforementioned tissues out to 168 h postdose at a low dose of 7.4 µg/kg *iv*. With increasing doses of 100 or 2000 µg/kg *iv*, with the exception of kidney, tissue levels declined rapidly compared to maximum levels (3 h postdose) but remained elevated above low levels confirming long residence time in selected tissues at saturation-type doses. Urinary excretion and blood cell distribution were also investigated. Urinary excretion increased dose-dependently, with very little of the LD recovered in urine, 0.93%, up to a maximum 20.32% at the HD. Blood cell distribution also varied with dose. Drug distributed to plasma at the LD and was approximately equally distributed to plasma and blood cells at HD, with the MD intermediate to LD and HD (higher in plasma than blood cells). The distribution data correlated well with DPP4 tissue expression and overall data were consistent with saturable, high affinity DPP4 binding of linagliptin.

Distribution trends were examined in DPP4-deficient Fisher rats (F344/DuCrlCrlJ) compared to wildtype (F344/DuCrl). Autoradiography trends in DPP-4 deficient rats

[11] Hong W et al. 1989. Exp Cell Res. 182:256-266

showed: (a) no biologically meaningful drug equivalents remained at 168 h postdose after either *iv* or oral treatment; (b) drug equivalents reached a maximum at 3 h postdose and at 24 h postdose drug equivalents were low in all tissues with the exception of liver (consistent with the common role of liver in metabolism); (c) testis concentrations were low compared to other tissues, but showed little elimination by 24 h postdose; and, (d) kidney drug equivalents were ≥ 20-fold higher in wildtype rats at ≥ 24 h postdose, supporting the role of DPP4-specific binding in kidney regions. Overall, trends showed apparent DPP4-specific binding in kidney outer medulla region as well as liver, lung, thymus, and epididymis, and possibly spleen and salivary gland. A separate study further examined PK trends, including the CD 1750 XX metabolite. At 'high' doses of ≥3 mg/kg, $t_{1/2}$ and V(ss) decreased in wildtype rats and $t_{1/2}$ increased slightly in DPP4-deficient rats. Thus, PK trends were nearly linear in DPP4-deficient rats and clearly non-linear in wildtype rats. Plasma $t_{1/2}$ trends were consistent with a high affinity, low capacity PK 'compartment' for drug, consistent with DPP4-specific binding or prolonged DPP4 interaction/affinity. CD 1750 XX metabolite PK trends were linear and essentially equivalent between wildtype and DPP4-deficient rats, with similar PK as parent in DPP4-deficient rats consistent with DPP4-independent kinetics. Overall, PK trends were consistent with the high affinity, low capacity DPP4-specific binding of BI 1356 BS suggested in knockout mouse studies. Tissue distribution trends were also consistent with other studies in DPP4-deficient rodents (Table 10). Drug was distributed preferentially to liver and kidney consistent with DPP4 distribution in wildtype rats. Tissue levels in DPP4-deficient rats were markedly lower than wildtype rats at low doses (≤ 1 mg/kg) and increased approximately dose-proportionally. **Data support the overall conclusion that DPP4 is a high affinity, low capacity compartment for BI 1356 BS, which is apparent at very low doses prior to DPP4 saturation (at which point distribution/accumulation seems due to DPP4 independent mechanisms). Binding to DPP4 is reversible but high affinity binding to BI 1356 BS results in prolonged $t_{1/2}$ in plasma and DPP4-containing tissues.**

### Table 10 – Linagliptin tissue distribution in wildtype and DPP4-deficient rats

Summary of tissue concentrations of radioactivity 72 h after intravenous (bolus) administration of [$^{14}$C]BI 1356 BS at various dose levels to DPP-IV deficient (def) or wildtype (WT) Fischer rats.

| dose [mg/kg] | strain | group | Kidneys [nmol/kg] | Liver [nmol/kg] | Lungs [nmol/kg] | Spleen [nmol/kg] | Subm. gland [nmol/kg] | Thymus [nmol/kg] | Skin [nmol/kg] |
|---|---|---|---|---|---|---|---|---|---|
| 0.01 | WT | 12 | 485 | 215 | 123 | 45.5 | 39.2 | 29.1 | 7.54 |
| 0.1 | def | 3 | 8.27 | 33.2 | 1.26 | 5.15 | 2.47 | 2.92 | 0.748 |
| 0.1 | WT | 4 | 3760 | 528 | 390 | 157 | 99.9 | 185 | 43.6 |
| 0.3 | def | 5 | 28.2 | 95.9 | 3.78 | 11.4 | 6.54 | 3.36 | 1.4 |
| 0.3 | WT | 6 | 3990 | 608 | 467 | 204 | 127 | 259 | 51.3 |
| 1 | def | 13 | 106 | 296 | 14.1 | 34.4 | 10.9 | 9.01 | 5.24 |
| 1 | WT | 14 | 4120 | 816 | 525 | 233 | 160 | 251 | 53.0 |
| 1 | def (old) | 1 | 122 | 307 | 26.9 | 135 | 36.8 | 24.3 | 5.58 |
| 1 | WT (old) | 2 | 3380 | 874 | 591 | 399 | 151 | 282 | 43.8 |
| 1 | WT pretr. | 18 | 660 | 368 | 111 | 85.6 | 82.5 | 96.7 | 16.6 |
| 3 | def | 15 | 364 | 806 | 45.1 | 124 | 47.4 | 24.2 | 13.1 |
| 10 | def | 9 | 1180 | 2220 | 154 | 594 | 264 | 157 | 53.8 |
| 10 | WT | 10 | 5310 | 2810 | 929 | 862 | 485 | 415 | 115 |
| 50 | def | 16 | 5030 | 9290 | 888 | 2780 | 700 | 605 | 312 |
| 50 | WT | 17 | 9280 | 8920 | 1960 | 3320 | 1020 | 918 | 299 |

DPP4 knockout and wildtype C57BL/6J tissue distribution and PK were assessed in selected tissues after a single low *iv* dose (2.12 µmol/kg). Distribution trends confirmed higher exposure and longer residence time in DPP4-containing target tissues. Total exposure differences were clear, with AUC approximately 10-fold higher in kidney and 3-fold higher in liver, skin, and lungs of wildtype mice compared to DPP4 knockouts. DPP4-specific binding in the terminal elimination phase (> 24 h postdose) was approximately 96% in kidney and 78% in liver (see reviewer's summary, Table 11). Lower DPP4-bound drug equivalents in liver may be due to liver-specific metabolites (e.g. inactive metabolites and/or metabolites that do not bind DPP4). Interestingly, tissue half-lives were very long in DPP4 knockouts (> 200 h) even though there was no potential DPP4-specific binding. Tissue half-lives were nearly 100 h longer (~25% longer) in kidney and lungs of DPP4-containing wildtype mice, while skin half-lives were equivalent. In contrast, liver half-life was approximately 25% longer in DPP4 knockout mice. The data provided insight into two important points with regards to long $t_{1/2}$ in DPP4 knockouts: (1) absolute tissue concentrations of radiolabel were markedly lower in DPP4 knockouts at distal time points, so while $t_{1/2}$ was long, tissue concentrations were very low; (2) the non-extractable fraction of radiolabel in liver and kidney was constant over time in wildtype mice (1-3%) but markedly increased with time in DPP4 knockouts (20% liver, 58% kidney), which contributed to prolonged tissue residence. Tissue distribution over time is shown, sexes combined, in the Sponsor's figure (Figure 5). Tissue distribution from a similar study in DPP4 knockout mice treated with linagliptin *iv* doses (0.01 – 10 mg/kg / 0.0212 – 106 µmol/kg) representing a wide range expected to provide sub-efficacious to saturating linagliptin concentrations is summarized in Table 12.

**Table 11 – DPP4-specific binding summary in mouse**

| DPP4-specific binding in kidney and liver | | | | |
|---|---|---|---|---|
| **Tissue** | **Postdose (time)** | **Tissue-specific drug equivalents** | | |
| | | **Administered dose (%)** | **Dose bound to DPP4 (%)** | **DDP4-specific binding (%)** |
| **Kidney** | 24 h | 1.7 | 1.6 | 94.5 |
| | 72 h | 1.3 | 1.2 | 95.9 |
| | 168 h (7 d) | 1.0 | 0.9 | 97.3 |
| | 672 h (28 d) | 0.3 | 0.3 | 96.6 |
| | **Mean** | -- | -- | **96%** |
| **Liver** | 24 h | 3.0 | 2.1 | 69.7 |
| | 72 h | 2.1 | 1.7 | 85.0 |
| | 168 h (7 d) | 1.3 | 1.2 | 86.2 |
| | 672 h (28 d) | 0.3 | 0.2 | 69.9 |
| | **Mean** | -- | | **78%** |

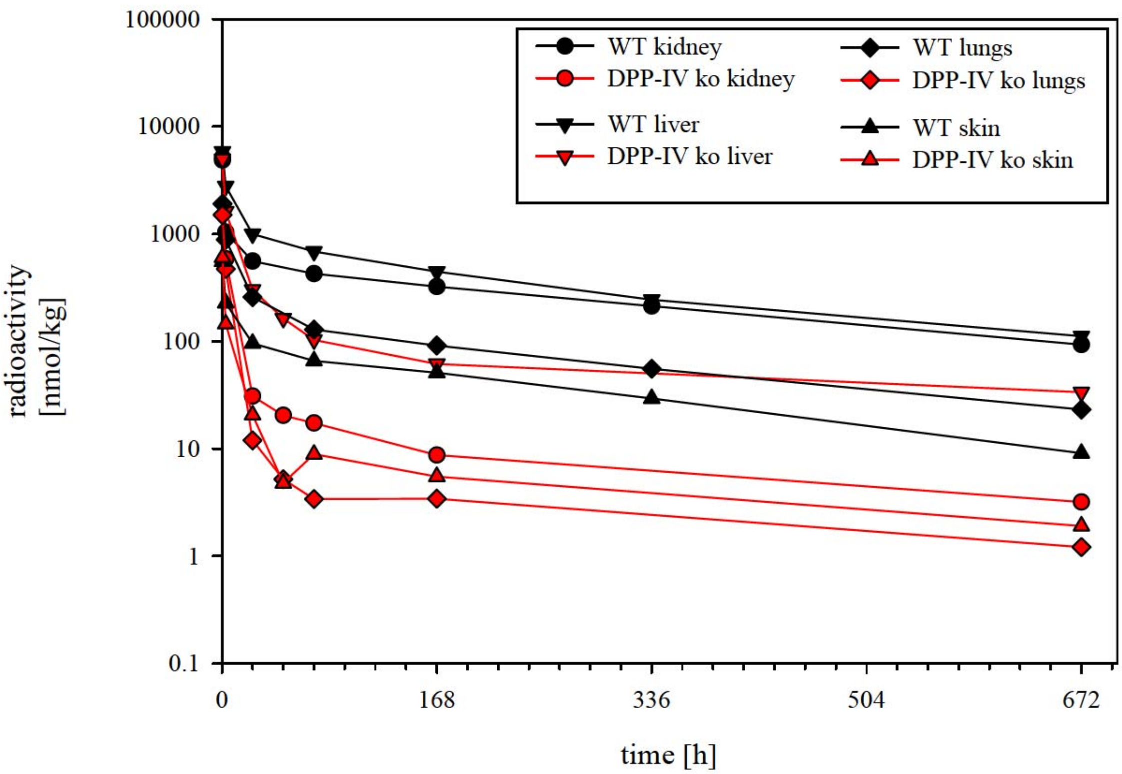



**Figure 5 – Tissue distribution time course in knockout and wildtype mice**

### Table 12 – Linagliptin tissue distribution in DPP4 knockout and wildtype mice

Summary of tissue concentrations of radioactivity 72 h after intravenous (bolus) administration of [$^{14}$C]BI 1356 BS at various dose levels to female wildtype (WT) or DPP-IV knockout mice.

| | Mean tissue concentrations [nmol/kg] | | | | | |
|---|---|---|---|---|---|---|
| **dose (mg/kg):** | **0.01** | **0.1** | **0.3** | **1** | **3** | **10** |
| | **DPP-IV knockout mice** | | | | | |
| Liver | 2.59 | 7.38 | 18.9 | 70.5 | 176 | 580 |
| Duodenum | BLQ | 1.20 | 1.33 | 5.17 | 7.73 | 22.4 |
| Carcass | BLQ | 2.03 | 3.59 | 20.9 | 26.2 | 118 |
| Skin | 1.03 | 2.24 | 1.65 | 6.68 | 13.5 | 36.5 |
| Lungs | BLQ | NC | 1.80 | 2.91 | 10.7 | 43.5 |
| Spleen | BLQ | NC | 4.56 | 8.04 | 17.9 | 83.6 |
| Kidneys | BLQ | 1.19 | 3.29 | 10.2 | 26.4 | 145 |
| Thymus | BLQ | BLQ | 5.65 | 6.55 | 16.7 | 62.8 |
| Muscle | BLQ | BLQ | BLQ | 0.764 | 2.23 | 7.04 |
| | **Wildtype mice** | | | | | |
| Liver | 131 | 546 | 638 | 652 | 716 | 1350 |
| Duodenum | 11.9 | 102 | 92.4 | 133 | 162 | 190 |
| Carcass | 5.68 | 28.1 | 35.8 | 48.2 | 66.4 | 171.5 |
| Skin | 9.44 | 52.8 | 78.3 | 77.4 | 105 | 130 |
| Lungs | 37.3 | 131 | 145 | 113 | 176 | 273 |
| Spleen | 13.4 | 50.4 | 55.1 | 47.7 | 97.8 | NOR |
| Kidneys | 215 | 474 | 533 | 477 | 517 | 687 |
| Thymus | 14.7 | 111 | 133 | 129 | 150 | 240 |
| Muscle | 1.32 | 4.12 | 4.43 | 4.8 | 7.99 | 15.5 |

BLQ = below lower limit of quantification
NC = not calculated
NOR = no valid results
file: FU_tissue_A084_07FU.xls

Linagliptin localization in DPP4-containing tissues was investigated in a 'micro autoradiogaphy' experiment in male albino rats. Rats were treated *iv* with a single bolus dose of [$^{3}$H]BI 1356 BS to investigate distribution of drug and/or metabolites microscopically in kidney, liver, and small intestine. A low dose of 7.4 μg/kg was used to investigate time-dependent distribution at 2 min, 3 h, and 192 h postdose. Higher doses of 100 and 2000 μg/kg were also investigated only for kidney distribution at 3 h postdose. ***Kidney*** **–** Results showed time-dependent kidney trends. Drug equivalents were originally distributed in glomeruli at 2 min postdose, consistent with its filtration function. By 3 h postdose, drug equivalents were high in proximal portions of nephrons and still very high in glomeruli. At 192 h postdose, radioactivity was high in proximal tubules and present at low levels in rectal parts of proximal tubules, with little radioactivity remaining in the glomeruli. At higher doses, distribution trends were different in kidney, with a broader distribution and higher linagliptin concentrations in proximal tubules, medullary rays (outer stripe of outer medulla), and proximal straight tubules. ***Liver*** **–** Distribution in liver showed a 'retiform' pattern, with localization to acinus zone 1 around portal triads and limited amounts in bile ducts at 2 h postdose. Distribution was similar 3 h postdose and with limited amounts in hepatocytes. By 192 h postdose, radioactivity persisted at low levels in bile ducts and hepatocytes with somewhat decreased amounts in acinar/portal triad regions. ***Small Intestine –*** Distribution was generally limited to mucosa and villi, with low levels of transfer to submucosa. The time course showed drug equivalents on villi surface and in lamina propria within mucosal villi at 2 h postdose, with most radioactivity on villi surfaces and intestinal lumen at 3 h and only trace amounts remaining at 192 h postdose. Biological relevance of small intestine trends for oral exposure are not clear because of *iv* exposure route in the study.

Autoradiography in pregnant rats also confirmed linagliptin crosses placenta and exposes developing fetus, consistent with separate maternal transfer studies in rat and rabbit. The maternal tissue distribution was similar to non-pregnant rats. Only trace amounts of radiolabeled linagliptin were found in fetal heart and liver. Low levels in fetal liver are consistent with DPP4 protein trends of much lower DPP4 in fetal liver compared to maternal liver[12].

## Metabolism

Linagliptin is not extensively metabolized in animals or humans. The majority of administered linagliptin is excreted unchanged in feces. A single major metabolite, CD 1790, was identified in humans and animals. CD 1790 is found in human plasma at a maximum 13% of administered linagliptin dose. Linagliptin has a single chiral center and the drug substance is the R-enantiomer (b) (4) [redacted] pure. CD 1790 represents the R-enantiomer metabolite of linagliptin. CD 1750 XX is the racemic mixture of CD 1790 and the corresponding S-enantiomer, CD 1789. Because linagliptin is (b) (4) [redacted] pure R-enantiomer, the CD 1750 racemic mixture is essentially pure R-enantiomer, CD 1790. No evidence of chiral inversion was observed *in vivo* in humans or animals ((b) (4) [redacted] R-enantiomer in plasma) and inversion was minimal *in vitro* or under long term storage. The Sponsor sometimes used the metabolite CD 1790 and CD 1750 interchangeably and although they are technically distinct, for practical purposes they are identical. All other metabolites of linagliptin are considered minor (<10% administered dose in humans) and they are present at very low levels in animal and human plasma.

The qualitative pattern of linagliptin metabolism is similar in humans and different animal species. CD 1790 is produced in mouse, rat, rabbit, and monkey and its toxicity was adequately evaluated in toxicology studies. Neither CD 1790 nor any other metabolites were found to be pharmacologically active *in vitro* based on DPP4 inhibition. As noted above, CD 1790 showed very low potency and incomplete inhibition of DPP4, which is considered not pharmacologically relevant at therapeutic exposures.

Metabolism was studied in several dedicated studies. *In vitro* metabolism in human liver and kidney microsomes and human hepatocytes was very low (< 2% metabolism after

[12] *IBID*

90 min), but they implicated CYP3A4 as the predominant metabolizing enzyme. CYP 1790 was formed *in vitro* by CYP3A4-mediated hydroxyl substitution of the primary amine on the 3-amino piperidine moiety of linagliptin. An intermediate ketone structure, CD 10604, was identified in the metabolic scheme, but it is rapidly reduced to CD 1790.

Potential for linagliptin or CD 1790 to inhibit metabolizing enzymes was assessed *in vitro*. Linagliptin was a “poor to moderate” irreversible (mechanism-based) inhibitor of human CYP3A4 in liver microsomes, with a $K_i$ of 115 µM. Linagliptin did not inhibit any other CYP isozymes or induced CYPs 1A2, 2B6, or 3A4 *in vitro*. Linagliptin also inhibited monoamine oxidase B (MAO-B) mediated metabolism *in vitro* with a $K_i$ = 2.4 µM. CD 1790 competitively inhibited CYP2C9 ($IC_{50}$ = 15-20 µM) and showed mechanism-based inhibition of CYP3A4 in human liver microsomes. The Sponsor estimated the interaction potential of CD 1790 for CYP3A4 would decreased intrinsic clearance by only 10%. Due to the high potency DPP4 inhibition of linagliptin, resulting in clinical $C_{max}$ of 20 nM, the potential for *in vivo* CYP3A4, MAO-B, or CYP2C9 inhibition are minimal at therapeutic exposures.

Chiral invertants were not found in human plasma after a single 600 mg oral dose of linagliptin. Chiral inversion was also not apparent in rat (100 mg/kg), mouse (100 mg/kg), or monkey (5 mg/kg) plasma analyses. There was some evidence of limited linagliptin chiral inversion in rabbit plasma, with approximately 98.2% stereo selectivity (compared to 99.9% in other species). CD 1789, the S-enantiomer metabolite, was identified in rats (5.4%) and rabbits (13.7%), compared to < 0.01% in human and < 0.04% in monkey.

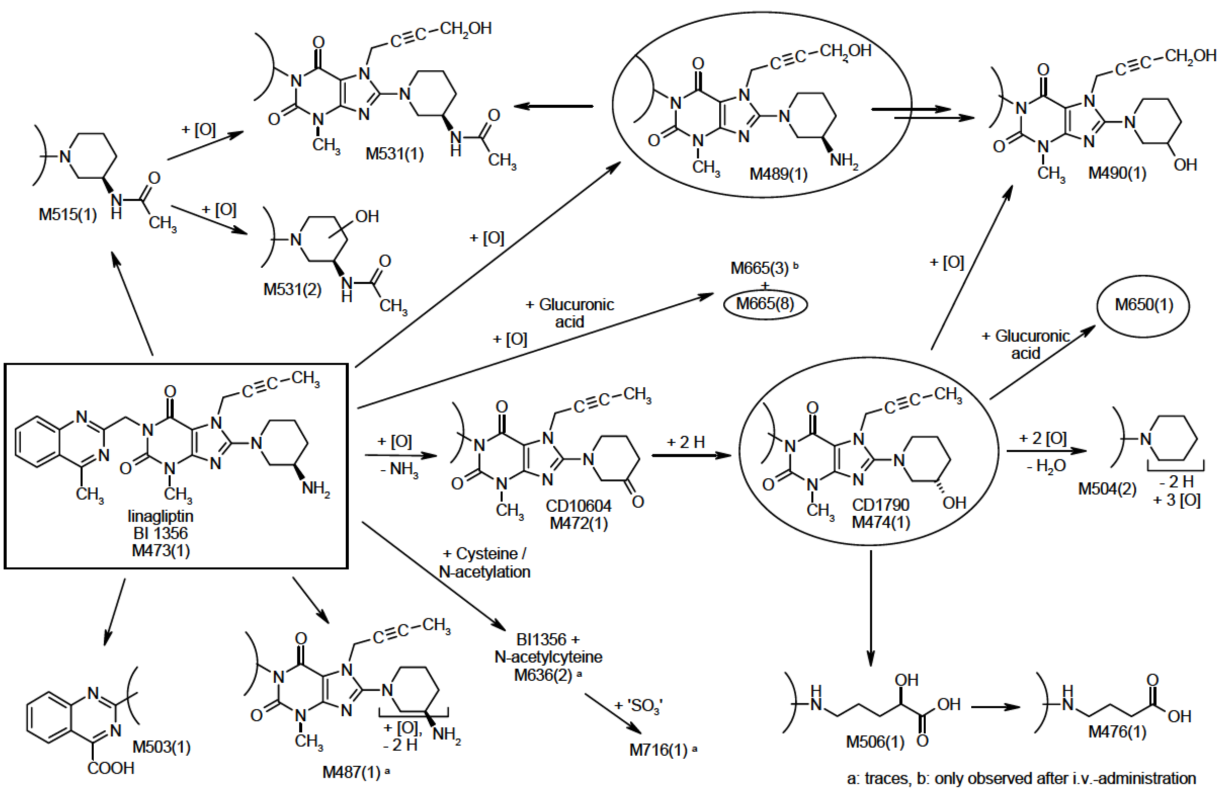



**Figure 6 – Linagliptin metabolic profile (human metabolites circled)**

## Excretion

Excretion and mass balance studies (some in conjunction with disposition/tissue distribution studies) were assessed in mice, rats, female rabbits, and monkeys. In humans and across species, linagliptin undergoes limited metabolism and is excreted predominantly as unchanged parent drug in feces. Approximately 80% of administered oral dose in humans is excreted unchanged in feces. The high affinity, low capacity DPP4-specific binding of linagliptin was shown to affect tissue distribution and excretion based on non-linear PK trends in animal models. The percentage of drug equivalents excreted in urine increased greatly when animals were treated with very high doses of linagliptin. At therapeutic doses, linagliptin plasma concentrations are in the range of DPP4-specific protein binding saturation, rather than greatly exceeding saturation as in nonclinical mass balance studies. Thus, clinical excretion trends are expected to be dominated by the fecal route.

Mass balance studies showed linagliptin fecal excretion ranged from 57% to 73% after *iv* dosing and higher fecal excretion, 67% to 95%, after oral dosing (Table 13). Fecal excretion trends were in the same range for humans. Urinary excretion after oral dosing was limited to 2% to 20% of administered oral dose in animal models and approximately 7% in humans. With *iv* dosing the contribution of urinary excretion was higher, ranging from 15% to 26% in animals. Urinary excretion after oral dosing was lowest in rat (2%)

and human (7%) and differences between *iv* and oral excretion trends were largest in the rat and humans. Trends under steady state conditions were assessed in rats treated with 2 mg/kg linagliptin for 14 days, with 91% fecal and 1% urinary excretion similar to single dose findings in rat.

**Table 13 – Sponsor's excretion summary**

Overview of the excretion balance of [14C]linagliptin related radioactivity in various species at selected doses (excretion data are given as % of dose)

| Species (Strain) | Mouse (CD-1) | | Rat (Wistar) | | Rabbit (Himalayan) | Cynomolgus monkey | | Human | |
|---|---|---|---|---|---|---|---|---|---|
| Route | i.v. | p.o. | i.v. | p.o. | p.o. | i.v. | p.o. | i.v. | p.o. |
| Dose [mg/kg] | 4 | 25 | 1 | 1 | 25 | 1.5 | 5 | 5 mg/ subject | 10 mg/ subject |
| Faecal excretion | 66.1 | 69.2 | 72.8 | 95.1 | 66.7 | 56.6 | 70.0 | 58.5 | 83.8 |
| Biliar excretion | ND | 46.2* | ND | 37.5(i.d.) | >5# | ND | 27.1 (i.d.) | ND | ND |
| Urinary excretion | 25.6 | 20.7 | 21.7 | 1.6 | 18.1 | 15.3 | 11.1 | 30.4 | 6.6 |
| Ae | 23.1§ | 18.4§ | 20.1§ | 1.4§ | 13.6 | 11.2 | 5.3 | 21.2 | 2.4 |

* maximum value (N=1)
\# biliary excretion not assessed quantitatively, value based on the maximum value (N=1)
Ae = amount excreted unchanged with urine
§ = estimated using the fraction of parent compound as determined in the respective in vivo metabolism studies
ND = not determined

Biliary excretion was variable across species. A study in cannulated rats treated *iv* with co-administration with (or without) a P-gp inhibitor showed biliary excretion plays a major role in linagliptin excretion. P-gp also played a significant role in biliary excretion, as evidenced by P-gp inhibition reducing biliary excretion of unchanged parent drug from 8.2% down to 3.2% of administered dose within 6 h post-dose. Metabolite biliary excretion was unaffected by P-gp inhibition and accounted for approximately 30% of administered linagliptin, although terminal half-life of parent and metabolite increased from 2-3 h to 7-11 h when P-gp was inhibited. Potential for enterohepatic circulation was investigated separately in rats and shown to be minimal. Gastrointestinal absorption of linagliptin dissolved in bile was very low in rats (2%), which may account for low enterohepatic circulation (estimated at 15%). In monkeys, intraduodenal administration of [$^{14}$C]-linagliptin resulted in 27% of the 5 mg/kg administered dose excreted in bile within the first 6 h, showing a significant role for biliary excretion in monkeys. In summary, biliary excretion seems to play a role in fecal excretion but does not seem to result in significant reabsorption of drug through enterohepatic circulation.

Linagliptin plasma PK, urinary excretion, and tissue distribution trends were also investigated in wildtype and DPP-4 knockout mice. Plasma PK and urinary excretion were investigated after bolus *iv* doses of 1 and 10 mg/kg BI 1356 BS. Plasma $C_{max}$ and AUC trends were generally equivalent (approximately ≤ 20% differences) in wildtype and DPP4-knockout mice. In contrast, plasma $t_{1/2}$ was approximately 10-fold shorter and volume of distribution (V(ss)) was markedly (4- to 23-fold) lower in DPP4-knockout mice. Plasma $t_{1/2}$ trends were consistent with a high affinity, low capacity PK 'compartment' for drug, consistent with DPP4-specific binding or prolonged DPP4 interaction/affinity. Urinary excretion trends were consistent with high affinity DPP4 binding at low doses (≤ 0.1 mg/kg), with saturation of DPP4 binding at ≥ 0.3 mg/kg. Urinary excretion of approximately 20% of administered dose was consistent at all doses in DPP4-knockout mice and at saturating linagliptin doses in wildtype mice. PK and urinary excretion data show non-linear PK trends in wildtype mice, consistent with DPP4-binding, while PK trends were linear in DPP4-knockout mice.

Excretion trends were assessed after multiple oral linagliptin administration in wildtype male and female rats. Rats were treated orally with [$^{14}C$]BI 1356 BS, 2 mg/kg/day for up to 14 days. The excretion profile was not affected by multiple dosing and reached an apparent steady state within one day after dosing. Feces was the major route of excretion, with approximately 90% of drug equivalents recovered in feces and only 1% in urine. By 24 h after the final dose approximately 91% of the total dose had been excreted with only 0.56% of total radioactivity remaining in carcass and tissues.

**Other Pharmacokinetic Studies**

Exposure to BI 1356 and metabolites was modeled with data from a single *iv* dose study in wildtype and DPP4-deficient rats. The PK model was intended to help predict drug exposure and time course in plasma, incorporating the influence of drug binding to DPP4 in plasma and tissues. Data from low dose exposures (0.01, 0.1, 0.3, and 1 mg/kg BI 1356) was used to design and test the model at non-DPP4-saturating concentrations of drug. The Sponsor's final model incorporated a 3-compartment design to account for DPP4-specific binding in plasma (peripheral) and tissue (central) compartments. The final model predicted similar affinity constant ($K_{on}/K_{off}$ = 231 pM) and binding site concentrations (2.7 nmol/l) as predicted by independent methods (e.g., plasma protein binding data). The model confirmed the experimental data for a reversible, high affinity, low capacity binding of BI 1356 by plasma and tissue DPP4, which drives linagliptin PK. A diagram of the model is shown in Figure 7.

**Figure 7 – Sponsor's final PK model representation**

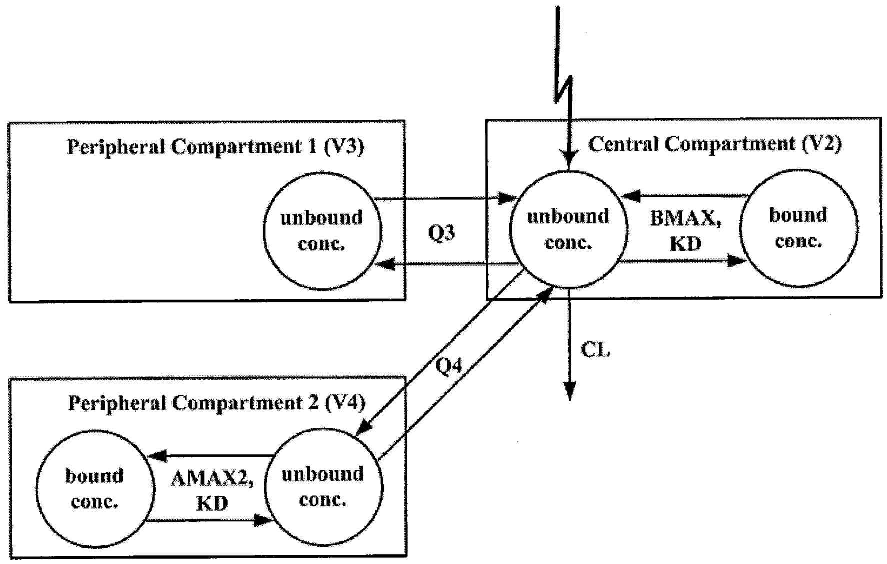



Figure 3.2.2:1 Schematic representation of the final PK model. Please note that bound conc. refers to BI 1356 BS bound specifically to DPP-IV and unbound conc. refers to the free BI 1356 as well as to BI 1356 which is bound non-specifically and non-saturable to proteins.

## 5.2 Toxicokinetics

**Maternal transfer and embryo-fetal exposure in rats and rabbits**
*Study reports 09B138 (U10-1332-01) and C73207 (U10-1684-01)*

Satellite or additional studies were conducted in pregnant rats and rabbits to assess maternal transfer of drug and major metabolite CD 1750 (rabbit only) to embryos/fetuses during gestation. Rat and rabbit doses were equivalent to MD and HD doses used in pivotal embryofetal development studies (Segment 2). Data are summarized below, which confirmed embryofetal exposure and showed developing rats were exposed to a greater percentage of maternal dose than rabbits. Sponsor's summary tables are also shown below.

***RAT –*** pregnant dams were dosed by oral gavage from GD 7 to GD 16 with 0, 30, or 250 mg/kg/d BI 1356. Direct fetal exposure was documented by fetal plasma detection, confirming drug crossed the placenta. Fetal exposure increased with dose and exposure compared to dams increased at the HD. Fetal exposure attained 27-39% maternal $C_{max}$ and 43-54% maternal AUC.

***Rabbit*** –pregnant does were dosed by oral gavage from GD 6 to GD 18 with 0, 25, or 150 mg/kg/d BI 1356. Direct fetal exposure was documented by fetal plasma detection, confirming drug and metabolite CD 1750 crossed the placenta. Fetal exposure increased with dose and exposure compared to does increased at the HD. Fetal exposure attained 2-5% maternal $C_{max}$ and 2-4% maternal AUC for linagliptin and 17-28% maternal $C_{max}$ and 15-24% maternal AUC for metabolite CD 1750.

### Sponsor's fetal rat exposure summary

Absolute and relative exposure in rat embryos and dams on gestation Day 16 after once daily oral (gavage) administration starting on gestation Day 7

| Parameter | 30 mg/kg | | | 240 mg/kg | | |
|---|---|---|---|---|---|---|
| | maternal | embryonal | ratio embryonal /maternal (%) | maternal | embryonal | ratio embryonal/ maternal (%) |
| **C(max) [nmol/L]** | 1960 | 526 | **26.8** | 13000 | 5050 | **38.8** |
| **AUC(0-24h) [nmol·h/L]** | 10600 | 4560 | **43.0** | 146000 | 79000 | **54.1** |

### Sponsor's fetal rabbit exposure summary

Mean toxicokinetic parameters of BI 1356 and CD 1750

| | | | BI 1356 | | CD 1750 | |
|---|---|---|---|---|---|---|
| **Parameter** | **Gestation Day** | **sample** | **25 mg/kg** | **150 mg/kg** | **25 mg/kg** | **150 mg/kg** |
| **C(max) [nmol/L]** | 6 | maternal | 5070 | 24900 | 268 | 988 |
| | 18 | maternal | 5680 | 33200 | 244 | 1500 |
| | | fetal | 96.5 | 1690 | 41.6 | 412 |
| | | ratio (%) fetal/maternal | **1.7** | **5.1** | **17.0** | **27.5** |
| **AUC(0-24h) [nmol·h/L]** | 6 | maternal | 18000 | 231000 | 798 | 8170 |
| | 18 | maternal | 20600 | 371000 | 787 | 13900 |
| | | fetal | 518 | 16300 | 119 | 3290 |
| | | ratio (%) fetal/maternal | **2.5** | **4.4** | **15.1** | **23.7** |

# 6 General Toxicology

## 6.1 Single-Dose Toxicity

### Single dose studies

Several non-pivotal, MTD, and dose-ranging single dose studies were conducted with linagliptin. Summaries of single dose toxicity studies are reproduced below.

**Single-dose Toxicity Study with Intravenous Administration in Mice and Rats**

| **DURATION** | 14 days | 14Days |
|---|---|---|
| Strain | Crl:NMRI mice | CrlGlxBrlHan:WI rats |
| No/Sex/Group | 3 | 3 |
| Route & Volume | IV&10ml/kg | IV&10ml/kg or 20ml/kg (for dose 120m/kg) |
| Daily Dose | 10, 30, 60mg/kg | 10, 30, 60, and 120mg/kg |
| Mortality | None | 2 Fs died immediately after administration at dose of 120mg/kg. High dose of 120mg/kg was not performed in other rats |
| Clinical Signs | None | None at doses up to 60mg/kg; None in 2 Fs that died |
| Body weight | No effects | No effects |
| Food effects | Not measured | Not measured |
| Hematology | Not measured | No drug related changes |
| Biochemistry | Not measured | Not measured |
| Urinalysis | Not measured | Not measured |
| Organ Weight | Not measured | Not measured |
| Necropsy findings | No positive findings | No positive findings at doses up to 60mg/kg; Dark red discoloration of the lungs and statis of the liver in 1 dead F, and stasis of the kidney and dark red discoloration of the jejunum in another one. |
| Histopath. | Not measured | Not measured |
| MNLD | 60mg/kg/day | 60mg/kg |

**Single-dose Toxicity Study with Oral Administration in Mice and Rats**

| **DURATION** | 14 days | 14Days |
|---|---|---|
| Strain | Crl:NMRI mice | CrlGlxBrlHan:WI rats |
| No/Sex/Group | 3 | 3 |
| Route & Volume | Oral gavage &10ml/kg | Oral gavage & 10ml/kg |
| Daily Dose | 1000 or 2000mg/kg | 1000 or 2000mg/kg |
| Mortality | 1M died about 4h post dose at 1000mg/kg | None |
| Clinical Signs | Reduced motor activity and pilorection in all animals, returned to normal in 1-2 days at 1000mg/kg and 7 days at 2000mg/kg | Slightly reduced motor activity and piloerection, returned to normal about 8hrs at 1000mg/kg, and 3 day at 2000mg/kg |
| Body weight | No effects | No effects |
| Food effects | Not measured | Not measured |
| Hematology | Not measured | No drug related changes |
| Biochemistry | Not measured | Not measured |
| Urinalysis | Not measured | Not measured |
| Organ Weight | Not measured | Not measured |
| Necropsy findings | Stasis of liver, a aqueous fluid filling the stomach, a milk-like fluid filing the small intestine, and red discoloration of the duodenum in dead M. | Red discoloration of the thymus in one F at 1000mg/kg |
| Histopath. | Not measured | Not measured |
| MNLD | Not determined | 2000mg/kg |

**Monkey *iv* MTD summary:** Single male and female monkeys were administered BI 1356 by *iv* infusion (10 min) to assess tolerability and range-finding after short term administration. The study consisted of two phases. In **Phase 1**, monkeys were given a "staircase" of 3- or 4-day treatments of 1, 2.5, 10, 25, and 50 mg/kg/day. The 50 mg/kg exceeded the MTD based on shallow breathing, closed eyes, lethargy, unsteady gait, decreased activity, and skin reddening. **Phase 2** comprised a 2-week treatment at 40 mg/kg in a single male and a single female. Results showed clinical signs of underactive behavior and eyes half closed and reticulocyte counts increased 4- to 6-fold. Increased APTT (↑ 30-50%), but not PT, suggested a possible treatment effect on blood clotting. Plasma histamine was measured to investigate potential for a pseudoallergy-type hypersensitivity response (seen in minipigs and dogs) but no treatment-related changes were seen. **The 40 mg/kg dose was considered tolerable for a subsequent 2-week study.**

## 6.2 Repeat-Dose Toxicity

**Dose-ranging studies**

Several 1- to 4-week dose-ranging toxicity studies were conducted in various animal models. Both dietary (palatability) and gavage oral range-finding studies were conducted. Palatability studies in rodents showed low exposure compared to gavage, with dosing accuracy generally confirmed up to about 500 mg/kg but not consistent at higher dietary concentrations. Because of problems with dosing and, more critically, low exposures achieved compared to gavage administration, dietary exposures were considered not feasible. Range-finding oral gavage toxicity studies in mouse, rat, dog, minipig, and monkey are summarized below.

<u>Mouse</u>

Mice were treated with up to 600 mg/kg linagliptin for 4- to 13-weeks. Toxicity and exposure trends were used primarily to identify appropriate doses for chronic carcinogenicity assessment. Chronic mouse toxicity, including neoplastic potential, is discussed in the carcinogenicity section below (Section 8).

**4-Week Mouse Key Study Findings:**

- There were no treatment-related mortalities.
- Stomach was the target organ. The MTD was 300 mg/kg/day based on the increased frequency and severity of histopathology findings in the non-glandular stomach at 600 mg/kg/day.
- The NOAEL dose was 60 mg/kg/day BI 1356 BS based on histopathology findings of hyperkeratosis or epithelial hyperplasia in the non-glandular stomach at 120 mg/kg/day.

3-Month Mouse
*GLP statement, 8/20/07*

**BI 1356 BS: Toxicity study by oral gavage administration to CD-1 mice for 13 weeks (BOI324/053238; Doc. No. U07-1536)**
*0, 100, 300, 600 mg/kg/d*
*75,200; 295,000; 521,000 nM*h*

*NOAEL = 100 mg/kg (476X MRHD)*

**Key Study Findings:**

- Clinical signs of toxicity were partially closed eyelids, piloerection, underactive behavior, and abdominal distension

- Abdominal distension was considered a symptom of decreased gastrointestinal motility, which, coupled with slightly decreased plasma glucose, were consistent with pharmacologic effects of DPP-4 inhibition due to increased circulating incretins (GLP-1, GIP).

- Stomach, liver, and kidney were identified as target organs

- MTD of 300 mg/kg/day was established based on treatment-related unscheduled deaths in the 600 mg/kg/day group attributed in several cases to renal and/or gastrointestinal toxicity (characterized by irritation-induced erosion which may have been exacerbated by decreased intestinal mobility)

- The NOAEL dose was 100 mg/kg/day based on findings of thickened keratinized mucosa, epithelial hyperplasia, and hyperkeratosis in the non-glandular stomach at 300 mg/kg/day

<u>Rat</u>

Toxicity was assessed in rats treated with 0, 6, 60, or 600 mg/kg/d for 4-weeks followed by a 4-week recovery period. The HD exceeded the MTD based on extensive toxicity and moribund sacrifice (3139-times MRHD). Toxicity in the HD was characterized by decreased BW and food consumption and target organ toxicity in intestine, liver, kidney, lymph nodes, thymus, lung, submandibular salivary gland, and spleen. foam cell accumulation indicative of phospholipidosis was seen in multiple organs. Toxicity was reversible with the exception of extrahepatic bile duct foam cells. A NOAEL of 60 mg/kg was determined (159-times MRHD). Additional toxicity details are summarized below.

**4-Week Rat – Summary of individual study findings “**In the 4-week rat general toxicity study with BI 1356 BS, doses of BI 1356 BS were tested up to 600mg/kg. One female in high dose group was sacrificed due to moribund condition. Clinical signs included piloerection, distended abdomen, emaciation, and focal alopecia at dose of 600mg/kg. These signs disappeared within 4 days of recovery. Decreased body weight was noted in females at doses of 60mg/kg and above and in males at dose of 600mg/kg in the treatment period. After 4-week recovery period, decreased body weight was still noted in females at dose of 600mg/kg. In addition, reversibly decreased food consumption was noted at 600mg/kg. Changes of clinical parameters included increases of white blood cells, neutrophils, lymphocytes, ALT, AST, GLDH, and aldolase in the treatment period and were reversible after the 4-week recovered period in high dose group. In addition, increased urinary inorganic material, protein and turbidities were noted at 600mg/kg in the treatment period and still cloudy with increased urinary protein at the end of recovery period. In the pathology study, drug-related changes of organ weights included increased kidney weights, decreased thymus, heart, prostate weights in males, and increased kidney, liver, adrenal weights, decreased thymus, heart, ovaries, and pituitary in females at dose of 600mg/kg at the end of treatment period. After 4- week recovery period, drug-related organ weight changes included increased kidney, liver and pituitary weights in males at dose of 600mg/kg were still observed. Drug-related macroscopic findings included light brown liver with multiple whitish and red discolorations in the sacrificed prematurely female, increased the contents and dilatation in large intestine in males at doses of 60mg/kg and above, and in females at 600mg/kg, reduction of intra-abdominal fat in females at doses of 60mg/kg and above, and in males at 600mg/kg, slightly to severely filled with very dry and firm ingesta in stomach, diffuse discoloration in liver, and focal alopecia in skin at 600mg/kg. After 4-week recovery period, no drug-related macroscopic findings were noted. In histopathology study, an extensive multifocal necrosis of the liver corresponded with the discoloration at necropsy was observed in the premature sacrificed female. The target organs in this animal is the same as other animals at dose of 600mg/kg including liver, kidneys, lymph nodes, thymus, lung, submandibular salivary gland, and spleen. The principal target organs of toxicity with BI 1356BS were intestine, liver, and kidney. The accumulation of foam cells in wide range of organs (lungs, lymph nodes, thymus, spleen, bone marrow and others) is indicative of phospholipidosis, which is well in accordance with the lipophilic cationic structure of BI 1356BS. There were no significantly drug-related findings at doses of 60mg/kg or less. At the end of recovery period, almost all findings were completely reversible except a minimal submucosal foam cells accumulation in the extrahepatic bile duct." **The NOAEL was 60mg/kg/day which occurred at 159X the MRHD.**

Non-rodent – Dog, Minipig, Monkey

Linagliptin toxicity in non-rodents was initially assessed in dog and minipig. A 2-week exploratory gavage study in dogs (0, 15, 45, 150 mg/kg) identified 45 mg/kg (242X MRHD) as the MTD based on moribund sacrifice of a HD male (pneumonia, myocardial necrosis at 1500-times MRHD) and liver, heart, lymph node, testes, lung, and bone marrow toxicity. A maximum of 45 mg/kg (242X MRHD) was tested in a 4-week dog study, which resulted in pseudoallergy with a positive histamine response and reversible liver, kidney, and testes toxicity. The NOAEL was 9 mg/kg (44-times MRHD). A comparative toxicity study was conducted in dog and Goettingen minipig, but minipigs also showed pseudoallergy response after one or two doses (2700-times MRHD) and were not considered the best non-rodent animal model for further toxicity studies.

Monkey was chosen for pivotal non-rodent toxicity studies, based on the pseudoallergy response seen in dogs and to investigate potential for necrotizing skin lesions that have been observed with other DPP4 inhibitors. No pseudoallergy response was observed in monkeys dosed orally up to 300 mg/kg/d for 4-weeks. A dose of 100 mg/kg was tolerated in a 2-week monkey study with toxicity limited to pancreatic acinar cell degranulation (NOAEL = 30 mg/kg, 226X MRHD). A dose of 300 mg/kg for 4-weeks exceeded the MTD based on kidney toxicity and moribund sacrifice, with kidney, lung, pancreas, adrenal, GI, gall bladder, thymus, lymph node, and female reproductive tract toxicity (>4000X MRHD). The 4-week monkey NOAEL was <10 mg/kg (66X MRHD) based on reversible lung alveolitis and pancreas islet congestion.

Toxicity in 2- and 4-week range-finding studies in non-rodents are described in slightly more detail in the summaries below.

**2-Week Oral Exploratory Gavage Toxicity Study in Dogs** (non-GLP)

| **DURATION** | 2 weeks |
|---|---|
| Strain | BASF beagle dogs |
| No/Sex/Group | 2 |
| Route & Volume | Oral gavage & 2ml/kg |
| Daily Dose | 0,15, 45, or 150mg/kg |
| Recovery | None |
| Mortality | 1M at 150mg/kg in moribund and sacrificed on Day 9 due to pneumonia and myocardial necrosis |
| Clinical Signs | Dose-dependent vomitus and pseudoallergic reactions including reddening and swelling of ears, circumocular region, upper lips) at ≥ 15mg/kg. Collapse at ≥ 45mg/kg |
| Body weight | No drug-related changes |
| Food effects | No drug-related changes except ↓ in the moribund animal |
| Ophthalmology | Not measured |
| EKG | A moderate, elevation heart rate at ≥ 15mg/kg and with shortening in PQ- and QT-intervals at 150mg/kg on Day 13 about 3.5h post dose. |
| Hematology | •Slight ↑ in the percentage of eosinophilc cells at 150mg/kg<br>•↓ Platelet in 1M at 150mg/kg |
| Biochemistry | •↑GLDH in 1M at 150mg/kg<br>•↓TG and cholesterol at ≥ 45mg/kg<br>•↑Plasma histamine at ≥ 15mg/kg |
| Urinalysis | No drug-related changes |
| Organ Weight | No-drug related changes |
| Necropsy findings | •In the moribund animal: slightly dilated heart with patchy pale discoloration of myocardium, and dark-red discoloration of lung lobes. In addition, pulmonary and costal pleura adhered to each other with visible fibrin fibers.<br>•Grey to red discoloration and consolidation of lung lobes at 15 and 45mg/kg, patchy grey discoloration of heart papillary muscle in 1F at 45mg/kg |
| Histopath. | •Liver: minimal to severe apoptosis of bile duct epithelial cells, minimal to slight amounts of mononuclear inflammatory cells infiltration in bile ducts, minimal to moderate diffuse vacuolization in bile ducts at ≥ 45mg/kg and scattered microgranuloma diffusely throughout the liver parenchyma in 1M at 150mg/kg.<br>•**Heart:** minimal to moderate hypertrophy in the media of myocardial arteries at ≥ 45mg/kg, severe disseminated myocardial necrosis in the moribund animal, and severe fibrosis of papillary muscle in one F at 45mg/kg.<br>•**Mesenteric lymph node:** slight accumulation of foam cells in the moribund animal with diffuse moderate vasculitis.<br>•**Male Genital system:** minimal to slight germ cell degeneration in testicular seminiferous tubules at ≥ 45mg/kg, minimal amount of cellular debris in epididymal duct in the moribund animal<br>•**Lung:** moderate to severe chronic interstitial pneumonia in 1F at 15mg/kg, 1M and 1F at 45mg/kg, and the moribund animal. May not be drug-related.<br>•**Bone marrow:** slight to severe increases of myelopoiesis/erythropoiesis ratio in Ms at 150mg/kg |
| TK | • System exposure increased in a more than dose proportional<br>• System exposure increased slightly on Day 12 than Day 1<br>• No gender difference |
| NOAEL | 15mg/kg with AUC 11900nmol.h/L (5623.9ng.h/ml) |
| Safety margins | 14 times based on AUC |

**4-Week Dog Summary:** "In the 4-week dog toxicity study, drug-related clinical findings included vomitus at doses of 9 mg/kg and above and pseudoallergic reactions at dose of 45mg/kg correlated with the increased plasma level of histamine. In the cardiovascular study, moderate hypotensive and tachycardic effect with a marginal trend of a QTc-prolongation was noted in high dose group and may be related to the pseudoallergic reactions. Changes of clinical chemistry parameters were noted in high dose group including increased ALT, GGT, GLDH and globulin in males. In the pathology study, no drug-related changes of organ weight and macroscopic findings. Drug-related histopathology findings were mainly in high dose group including minimal, focal, subacute periductal inflammatory infiltration of large intrahepatic bile ducts in females; delicate foci of tubuloepithelial apoptosis/necrosis with minimal granulocytic infiltration in the kidney in one male; and slight focal atrophy in seminiferous tubules in testes in two males. All of these findings were reversed after 4-week recovery period. The **NOAEL was 9mg/kg**." The AUC of approximately 7000 nmol*h/l provided a safety margin of 44-times the MRHD.

**2-Week Monkey Summary:** "Administration of BI 1356BS to cynomolgus monkeys resulted in no clinical signs indicative of pseudo-allergy as found in dog study. There were no drug-related changes of body weight, EKG, blood pressure and pulse rate, hematology, clinical chemistry and urinalysis. In the pathology study, no drug-related changes in organ weights and macroscopic findings. In the histopathology exam, minimal acinar cell degranulation in pancreas was found in one male and two females at dose of 100mg/kg at the end of treatment period. This change was reversible. There were no other drug-related findings were observed at the end of treatment period or recovery period. The **NOAEL was 30mg/kg** with AUC of 35,650 nmol*h/l." The safety margin was 226-times the MRHD.

**4-Week Monkey Summary:** Cynomolgus monkeys were treated for 4-weeks with 0, 10, 60, and 300 mg/kg/d linagliptin by oral gavage followed by a 4-week treatment-free period. The NOAEL was considered <10 mg/kg based on reversible lung alveolitis (males) and pancreas islet congestion (females). The MTD was 60 mg/kg based on renal toxicity and moribund sacrifice in males. Other potential target organs included adrenals, jejunum, kidney, gall bladder, thymus, lymph node, and female reproductive tract. Most findings were reversed after the drug-free recovery period.

**Key Study Findings:**

- One male 300 mg/kg HD was sacrificed in moribund condition on day 8 with death attributed to kidney toxicity
- Lung histopathology findings suggestive of potential phosholipidosis included reversible alveolar macrophage and perivascular inflammatory cell infiltration, and alveolitis.

## Pivotal repeat-dose toxicity studies

Pivotal sub-chronic and chronic oral toxicity studies were conducted in rats and monkeys. Rats were treated for 3- and 6-months in standard toxicity studies and for essentially lifetime exposure over 2-years in a carcinogenicity study. Linagliptin exposure in the 6-month rat study covered a range of 10- to >1700-times clinical exposure. Rat NOAELs determined in the 6-month (30 mg/kg) and 2-year study (18 mg/kg for non-neoplastic findings) provided safety margins of 66-times and 51-times clinical exposure, respectively. Monkeys were treated for 3- and 12-months and linagliptin was tolerated in most monkeys up to 100 mg/kg (791X MRHD) for a year, although one 100 mg/kg female monkey died with signs of kidney failure. A NOAEL of 10 mg/kg linagliptin was determined for chronic exposure in monkeys providing a safety margin of 40-times clinical exposure. Detailed summaries of pivotal rat and monkey toxicity studies are shown below.

### 3-Month Rat (sub-chronic)

*GLP statement, initiated 7/5/06*

**BI 1356 BS: Toxicity study by oral gavage administration to Han Wistar rats for 13 weeks followed by a 6 week recovery period (BOI323/053709; Doc. U06-1301)**
*0, 10, 30, 100, 300 mg/kg/d*
*2050; 15,150; 79,400; 238,000 nM*h*

*NOAEL <10 mg/kg (<13X MRHD multiples)*

**NOAEL determination –** No NOAEL was determined based on minimal to slight alveolar macrophage aggregation, suggestive of phospholipidosis, in all treatment groups. The HD (1506X MRHD) exceeded the MTD based on markedly decreased BW gain and mortality that could not be ruled out as related to drug treatment. A NOAEL was determined in the 6-month chronic rat study described below.

**Key Study Findings:**

- There were 3 unscheduled deaths. The sponsor considered the deaths of 2 males in the high dose 300 mg/kg/day BI 1356 BS group (one main study, one TK satellite group) unrelated to treatment, but a treatment related cause was not ruled out and both had brain hemorrhages. A female in the 100 mg/kg/day TK group died on the first day of dosing.

- The MTD in males was 100 mg/kg/day BI 1356 BS based on mortality and 24% decreased body weight gain compared to control at 300 mg/kg/day. In females, a MTD was not established up to 300 mg/kg/day.

- The NOAEL dose was < 10 mg/kg/day in both males and females based on histopathology findings of minimal/slight aggregations of alveolar macrophages in lungs at 10 mg/kg/day and above.

- Findings of aggregations of alveolar macrophages in lungs and cationic structure of BI 1356 BS are consistent with phospholipidosis.

- Target organs of toxicity were lung, liver, thyroid (females), and uterus.

- BI 1356 BS was found in the blood of control group rats. Because drug contamination primarily occurred at 2 time points (8 hour on day 1 and 1 hour on day 85), plasma levels were substantially lower than levels from any time point from the low dose group, and drug was not detectable prior to dosing in any control rat, the sponsor believes samples were contaminated ex vivo, probably during the plasma separation phase, and does not reflect the level of drug in control rats.

## 6-Month Rat (chronic)

*GLP statement, 2/6/08*

**BI 1356 BS: 26-Week oral (gavage) toxicity study in rats (05B285; Doc. No. U07-1910)**
*0, 7, 30, 100, 300 mg/kg/d*
*1542; 10,400; 54,650; 282,000 nM*h*

*NOAEL = 30 mg/kg (66X MRHD multiples)*

**NOAEL determination –** Toxicity was generally similar at HMD and HD, with dose-related toxicity in kidney, liver, lung, stomach, thyroid, and liver. The HD led to moribund sacrifice of one male rat. General toxicity or possibly neurotoxicity presented as transiently decreased locomotor activity. Findings are summarized in the reviewer's summary table below.

**26-Week Repeat Dose Toxicity in Rat — Summary**

| SPECIES<br>DOSES AND ADMINISTRATION<br># ANIMALS<br>FOLLOW-UP | NOAEL = 30 MG/KG/DAY;<br>66x MRHD |
|---|---|
| Wistar rat (Crl:WI(Han))<br>26 week + 8-week recovery<br><br>0, 7, 30, 100, 300mg/kg/day<br>Oral gavage (Vehicle: 0.5% hydroxyethylcellulose, 10 ml/kg)<br><br>Main: 20/sex/group<br>Recovery: 10/sex (con., HD)<br>TK: 5/sex/group (days 1,88,178) | see AUC table below<br>NOAEL = 30 mg/kg/day (66x MRHD) |

| $AUC_{0\text{-}24h}$ (nM*h) | | | | |
|---|---|---|---|---|
| Dose (mg/kg) | Males | | Females | |
| | Day 1 | Day 178 | Day 1 | Day 178 |
| 7 | 464 | 816 | 428 | 762 |
| 30 | 6910 | 10600 | 9800 | 10200 |
| 100 | 37400 | 53900 | 63300 | 55400 |
| 300 | 99100 | 250000 | 217000 | 313000 |

NOAEL = 30 mg/kg/day (66x MRHD)

**Mortality:** Single moribund death 300 mg/kg/day (1/20 ♂). Three accidental deaths (gavage errors), not dose-related.

**Clinical Signs**: NOAEL = 30 mg/kg/day. At ≥ 100 mg/kg/day animals had ↓ locomotor activity for 5-6 h postdose, but reactions to cageside inspections were normal.

**Body Weight**: No treatment related findings.

**Hematology**: No treatment related findings.

**Clinical Chemistry**: NOAEL = 30 mg/kg/day. At ≥ 100 mg/kg/day findings included ↑ ALT (2 – 3x), ↑ glutamate dehydrogenase (GLDH; 3-25x ♂, 2-4x ♀), ↑ AST (>2x 300 mg/kg/day ♂).

**Organ Weights:** NOAEL = 30 mg/kg/day. Increased liver and kidney weights, minimal (<10-15%) at 100 mg/kg/day and 300 mg/kg/day ♂ and up to ↑18% (kidney) to 29% (liver) in high dose females.

**Gross Pathology**: NOAEL = 100 mg/kg/day. High dose discolored kidney (2/18 ♂, 2/20 ♀), lung focal discolorations (1/18 ♂), and stomach mucosal lesions (1/18 ♂, 3/20 ♀).

**Histopathology:** NOAEL = 30 mg/kg/day. Liver and kidney confirmed as target organs at ≥ 100 mg/kg and additional lung, stomach, and thyroid high dose findings: liver cytoplasmic rarefaction (glycogen accumulation), centrilobular hypertrophy, pigment storage (lipofuscin); kidney proximal tubular basophilia, convoluted tubule focal basophilic tubules, tubular pigment storage; lung foam cell accumulation (phospholipidosis, described as "very mild" by pathologist); stomach mucosal irritation; ovary, vagina, prostate alterations.

**Toxicokinetics:** Drug was readily absorbed after oral gavage. Plasma concentrations were slightly higher at steady state on day 88 compared to day 1. There were no apparent sex differences in exposure after repeated dosing.

**Summary**: NOAEL = 30 mg/kg/day. Toxicity was generally similar at 100 and 300 mg/kg/day, with severity increased at the high dose. General toxicity or possibly neurotoxicity manifest as transiently decreased locomotor activity. Target organs were kidney, liver, lung, stomach, and thyroid, with monitorable clinical biomarkers in liver.

## 3-Month Monkey (sub-chronic)

*GLP statement, 5/8/07*

**BI 1356 BS: Toxicity study by oral gavage administration to cynomolgus monkeys for 13 weeks followed by a 6 week recovery period (Study BOI315/052597; Doc. No. U07-1072-01)**
*0, 4, 25, 150 mg/kg/d*
*3325; 22,700; 279,500 nM*h*

*NOAEL < 4 mg/kg/d (21X MRHD)*

**NOAEL determination –** Linagliptin was generally well tolerated and the HD was considered below the MTD. However, no NOAEL was determined based on modest LD findings in liver, lung macrophages, periodontal gingivitis and adrenal focal mineralization and similar toxicity in higher doses that were not full reversed after a 6-week drug-free period. A NOAEL was determined in the 12-month chronic monkey study described below.

**Key Study Findings:**

- There were no unscheduled deaths during the study.
- The MTD was > 150 mg/kg/day BI 1356 BS in both male and female cynomolgus monkeys.
- The NOAEL was < 4 mg/kg/day BI 1356 BS based on liver cleft pale areas (males), pigment-laden macrophages in lung (females), periodontal gingivitis (females), and adrenal focal mineralization (females) at the lowest dose of 4 mg/kg/day.
- Target organs/tissues of toxicity were adrenal (females), kidney, liver (male), lung (females), ovaries, stomach, and teeth (females).

## 12-Month Monkey (chronic)

*GLP statement, 2/29/08*

**BI 1356 BS: Toxicity study by oral gavage administration to cynomolgus monkeys for 52 weeks followed by an 8 week recovery period (Study BOI 0331/072859; Doc. No. U08-1185-01)**
*0, 1, 10, 100 mg/kg*
*352; 6265; 125,000 nM*h*

*NOAEL = 10 mg/kg/d (40X MRHD)*

**NOAEL determination –** Drug was essentially tolerated up to 100 mg/kg/day, with the exception of 1/4 HD females euthanized in moribund condition with apparent kidney failure and decreased body weight gain in males. Additional findings included markedly reduced HD male BW gain and potentially delayed sexual maturation based on decreased reproductive organ weights. Findings are summarized in the reviewer's summary table below.

| 52-Week Repeat Dose Toxicity in Monkeys — Summary | |
|---|---|
| **SPECIES**<br>**DOSES AND ADMINISTRATION**<br>**# ANIMALS**<br>**FOLLOW-UP** | **NOAEL = 10 MG/KG/DAY;**<br>**40X MRHD** |
| Cynomolgus monkey<br>52-week + 8-week recovery<br><br>0, 1, 10, 100 mg/kg/day<br><br>Oral gavage (Vehicle: 0.5% hydroxyethylcellulose, 5 ml/kg)<br><br>4/sex/group<br>2/sex/group recovery (con., HD) | (see AUC table below)<br>NOAEL = 10 mg/kg/day (40x MRHD) |
| **Mortality:** 1/4 ♀ at 100 mg/kg/day, with cause of death consistent with kidney failure (nephrotic syndrome?) although the single kidney necropsied lacked histological lesions. | |
| **Clinical Signs**: Limited to postdose salivation and vomiting, predominantly at the high dose. Sporadic salivation at 1 and 10 mg/kg/day. | |
| **Body Weight**: NOAEL = 10 mg/kg/day. High dose male body weight gain markedly decreased, with rapid increased weight gain during the first two weeks of recovery. No apparent effect on female body weight gain. ***Food consumption –*** no apparent effects (visual assessment). | |

| $AUC_{0-24h}$ (nM*h) | | | | |
|---|---|---|---|---|
| Dose (mg/kg) | Males | | Females | |
| | Day 1 | Day 365 | Day 1 | Day 365 |
| 1 | 444 | 444 | 740 | 260 |
| 10 | 12000 | 5740 | 15500 | 6790 |
| 100 | 170000 | 128000 | 142000 | 122000 |

| |
|---|
| **ECG:** No treatment related findings. $QT_c$ was elevated in high dose males at 24 h postdose (but not 2 h postdose) during week 13 only, which was considered anomalous due to absence of findings at other times and in high dose females. |
| **Hematology**: No treatment related findings. |
| **Clinical Chemistry**: No treatment related findings. |
| **Organ Weights:** Indications of potential male and female delayed sexual maturation at 100 mg/kg/day, including ↓ prostate weight and trends of ↓ testis and ↓ epididymis weights (comparisons skewed by 1/4 control, 1/4 mid dose mature ♂); females had ↓ uterine weight (within the historical range), ↓ ovary weight (100 **and** 10 mg/kg/day, all near or within the historical range), and ↓ number of corpora lutea. |
| **Gross Pathology**: No treatment related findings. No evidence of skin lesions. |
| **Histopathology:** No notable treatment related findings. A single high dose female (1/4) had moderate islet cell proliferation in the pancreas, which the sponsor considered a response to DPP4 inhibitor treatment and consistent with improved pancreatic β cell mass and function seen in some diabetic animal models. |
| **Toxicokinetics:** Drug was readily absorbed after oral gavage ($T_{max}$ ≈ 0.5 – 4 h). Plasma exposure increased with dose, greater than dose proportionally from 1 to 10 mg/kg and approximately dose proportionally from 10 to 100 mg/kg. The major metabolite, CD 1750 XX, had similar $T_{max}$ and constituted approximately 20-30% of parent plasma drug equivalents (as $C_{max}$ or AUC) in all dose groups. There were no apparent sex differences in exposure. |
| **Summary**: NOAEL = 10 mg/kg/day. Drug was essentially well tolerated up to 100 mg/kg/day, with the exception of 1/4 ♀ euthanized in moribund condition with apparent kidney failure and decreased body weight gain in males. |

# 7 Genetic Toxicology

## 7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

**Ames assay**
*GLP statement, 10/12/04*

**BI 1356 BS: Mutagenicity study using the *S. typhimurium*/mammalian-microsome assay (Ames test) (04B074; Doc. No. U04-1756)**

**Key Study Findings:**

- No evidence of mutagenic potential in the presence or absence of a metabolic activation system up to bacteriotoxic concentrations of 1000 µg/plate

## 7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

**Chromosome aberration in HPBL cells**
*GLP statement, 10/11/04*

**BI 1356 BS: Mutagenicity study for chromosomal aberrations in human lymphocytes *in vitro* (04B069; Doc. No. U04-1827)**

**Key Study Findings:**

- No evidence of clastogenicity in primary human lymphocyte cultures in the absence or presence of a metabolic activation system up to concentrations that did not cause excessive cytotoxicity

## 7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

***In vivo* micronucleus assay in rat**
*GLP statement, 10/11/04*

**BI 1356 BS: Mutagenicity study using the micronucleus assay in rat bone marrow (Part of the 4-week oral (gavage) toxicity study (04B067; Doc. No. U04-1847)**

**Key Study Findings:**
- No evidence of clastogenic potential in rats administered up to 600 mg/kg linagliptin orally for 4-weeks

## 7.4 Other Genetic Toxicity Studies

### 7.4.1 Metabolites

**CD 1750 Ames assay (initial)**
*GLP-compliant, 4/4/06*

**CD 1750 XX (metabolite of BI 1356 BS): Mutagenicity study using the *S. typhimurium*/mammalian microsome assay (Ames test) (Study 05B282; Doc. No. U06-1188)**

**Key Study Findings:**

- CD 1750 XX at 30, 100, and 3000 µg/plate increased the number of revertant colonies above the historical control range in strain TA 98 without metabolic activation

- CD 1750 XX at 30, 100, and 3000 µg/plate increased the number of revertant colonies above the historical control range in strain TA 102 with metabolic activation

- The original reviewer considered the study invalid because:
    - The assay did not meet the sponsor's acceptance criteria. The number of revertant colonies in vehicle control plates were outside the historical range for *S. typhimurium* strains TA 98 (without metabolic activation) and TA 102 (with metabolic activation)
    - The positive control for TA 98 without metabolic activation, 2-nitrofluorene, increased the number of revertant colonies < 2-fold

**CD 1750 Ames assay (repeat)**
*GLP-compliant, 11/15/07*

**CD 1750 XX (metabolite of BI 1356 BS): Mutagenicity study using the *S. typhimurium*/mammalian microsome assay (Ames test) – Supplementary study (Study 07B046; Doc. No. U07-2080)**

**Key Study Findings:**

- No evidence of mutagenic potential in the presence or absence of a metabolic activation system up to the solubility limit of 3000 µg/plate

- This reviewer concludes the results of the repeat assay confirm the absence of CD 1750 mutagenic potential under the conditions of standard Ames mutagenesis assays

**CD 1750 Chromosome aberration assay**
*GLP-compliant, 7/10/06*

**CD 1750 (metabolite of BI 1356): Mutagenicity study for chromosomal aberrations in human lymphocytes *in vitro* (Study 05B283; Doc. No. U06-1585)**

**Key Study Findings:**

- Negative for clastogenicity in primary human lymphocytes in the presence or absence of a metabolic activation system up to concentrations that did not cause excessive cytotoxicity and/or exceed the solubility limit

### 7.4.2 Impurities

The Sponsor identified several impurities and/or degradants which required identification and potent qualification. As discussed in Section 2.5, above, all impurities and degradants were qualified in accordance with current guidance and none are considered to pose a significant toxicologic risk. Results of genetic toxicity evaluations are summarized in Table 14. Individual assays conducted to qualify potential genetic toxicity of impurities are reviewed or summarized in Appendix 1.

(b) (4)

[redacted]

# 8 Carcinogenicity

**Rat 2 year oral carcinogenicity study**

*0, 6, 18, 60 mg/kg/d (oral gavage)*
*1.5, 8, 66 µM*h BI 1356 BS (9X, 51X, 418X MRHD)*
*0.1, 1, 4 µM*h CD 1750 XX (metabolite)*

**Key Study Findings:**

*NOAEL (neoplastic) = 60 mg/kg (418X MRHD)*

*NOAEL (non-neoplastic) = 18 mg/kg (51X MRHD)*

Adequacy of Carcinogenicity Study – The final study report of a GLP-compliant, standard two year oral (gavage) carcinogenicity study in Han Wistar rat was reviewed and results were discussed at a meeting of the Executive Carcinogenicity Assessment Committee (ECAC). The study was considered acceptable based on doses previously recommended by the ECAC and which provide exposure greater than 25-times the expected maximum human dose. Satellite animals were included for toxicokinetic analyses and mouse exposures could be compared to expected human exposure.

Appropriateness of Test Models – The Sponsor chose doses of 0, 6, 18, and 60 mg/kg/day linagliptin (BI 1356 BS) based on previous recommendations of the ECAC. Treatment was well tolerated and results showed no dose-limiting toxicity up to the highest dose tested. Exposure at the high dose provided approximately 418X MRHD based on total exposure ($AUC_{0-24}$).

Evaluation of Tumor Findings – There were no tumor increases in any linagliptin treatment group that were considered treatment-related or biologically significant. Thyroid benign C-cell tumors seemed slightly elevated in male MD (24% incidence) and female LD (33% incidence) groups, but neither trend test for dose-response nor individual pair-wise comparisons were statistically significant for these common tumors. Furthermore, there were no increases in malignant C-cell tumors and there were no clear treatment-related trends in C-cell hyperplasia (which is considered an earlier sign along a progression from hyperplasia to benign and ultimately invasive malignant tumors). Background incidence of C-cell adenomas was also slightly higher in the current study and C-cell adenoma findings were considered spontaneous and not treatment related.

Summary of Non-neoplastic Findings – Lung cholesterol cleft granuloma(ta) incidence increased in male and female HD groups. Overall cholesterol cleft incidence were modest in HD males (27%) and females (27%) but trends in both sexes showed a drug-related HD increase compared to concurrent controls. Lesions suggesting drug-related lung phospholipidosis (e.g., foamy cell macrophages) were also seen in shorter duration rat studies and cholesterol cleft granuloma(ta) were consistent with sequelae secondary to lung phospholipidosis. The biological significance of chronic lung phospholipid findings is unclear based on the absence of a significant toxicological correlate (e.g., increased clinical signs, mortality, or evidence of progression to neoplasms). No other non-neoplastic lesions were considered clearly biologically significant or adverse. Kidney metastatic transitional epithelial mineralization and reactive transitional epithelial hyperplasia were increased in most treatment groups but there was a high background incidence in controls and no tumor response or other apparent treatment-related effect on chronic kidney function.

**Title – BI 1356 BS: Carcinogenicity study by oral gavage administration to Han Wistar rats for 104 weeks**

| | |
|---|---|
| Study no.: | BOI0332 (Doc. No. U10-1502-01) |
| Study report location: | eCTD 4.2.3.4.1 |
| Conducting laboratory and location: | [redacted] (b) (4) |
| Date of study initiation: | 6/28/06 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | BI 1356 BS, Batch No. 5060170, 97.7-97.9% purity |
| CAC concurrence: | Yes |

## Methods

| | |
|---|---|
| Doses: | 0, 6, 18, 60 mg/kg/d |
| Frequency of dosing: | QD |
| Dose volume: | 10 ml/kg |
| Route of administration: | Oral (gavage) |
| Formulation/Vehicle: | 0.5% aqueous hydroxyethylcellulose (Natrosol® 250 HX) |
| Basis of dose selection: | > 25X AUC at MRHD |
| Species/Strain: | Han Wistar rat (HsdHan™:Wist) |
| Number/Sex/Group: | 55 |
| Age: | 40-46 days (6-7 weeks) |
| Animal housing: | 5/sex/cage (4/sex/cage TK satellite) |
| Paradigm for dietary restriction: | None (*ad lib.* feeding except during urine collection & o/n fast prior to TK blood sampling) |
| Dual control employed: | No |
| Interim sacrifice: | No |
| Satellite groups: | 12/sex/group |
| Deviation from study protocol: | Several minor deviations that did not affect the validity of the study results were reported, including (but not limited to): HD Female #4F 491 was underfed up to 4% during weeks 21 to 24 due to missing BW recording at week 20; 20 hematology samples from unscheduled necropsies were analyzed > 2 d post-collection and they were not reported (b/c stability of samples > 2 d has not been established) |

### Study Design Summary

| Group | Treatment | Dosage# (mg/kg/day) | Main study No. of animals Male | Main study No. of animals Female | Main study Animal numbers Male | Main study Animal numbers Female | Satellite study† No. of animals Male | Satellite study† No. of animals Female | Satellite study† Animal numbers Male | Satellite study† Animal numbers Female |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Control | 0 | 55 | 55 | 1-55 | 269-323 | 12 | 12 | 56-67 | 324-335 |
| 2 | BI 1356 BS | 6 | 55 | 55 | 68-122 | 336-390 | 12 | 12 | 123-134 | 391-402 |
| 3 | BI 1356 BS | 18 | 55 | 55 | 135-189 | 403-457 | 12 | 12 | 190-201 | 458-469 |
| 4 | BI 1356 BS | 60 | 55 | 55 | 202-256 | 470-524 | 12 | 12 | 257-268 | 525-536 |

# Expressed in terms of test material as supplied.

† Satellite animals used for toxicokinetic sampling only

<u>Observations and Results</u>

***Statistics* –** Statistical analyses were conducted by the Sponsor and independently by the FDA. Statistical trend test for dose response and pair-wise test for differences between individual treatment groups and controls were conducted for mortality and tumor incidence. The Sponsor followed international guidance for tumor analyses, considering “common” tumors (> 1% historical incidence) significant at p< 0.005 and p< 0.01 and "rare" tumors (< 1% historical incidence) significant at p< 0.025 and p< 0.05, for trend and pair-wise tests, respectively.

The Sponsor considered the following tumors to be "rare" in their analysis (with all others considered "common"):

**Males**

Parathyroids - Benign chief cell adenoma
Skeletal Muscle - Benign haemangioma
Skin - Malignant squamous cell carcinoma
Skin - Benign squamous cell papilloma and malignant squamous cell carcinoma combined
Skin - Benign basal cell tumour
Brain - Benign granular cell tumour

**Females**

Skin - Benign keratoacanthoma
Skin - Benign squamous cell papilloma and malignant squamous cell carcinoma combined
Brain - Benign granular cell tumour
Uterine cervix - Malignant schwannoma
Clitoral Glands - Malignant squamous cell carcinoma
Clitoral Glands - Benign squamous cell papilloma and malignant squamous cell carcinoma combined

There were no statistically significant increases in dose-related trends or in individual treatment group pair-wise analyses.

***Mortality –*** There was no apparent treatment effect on survival. Overall survival was 62-69% in males and 58-71% in females. Mortality was 5-7% higher (nss) in MD and HD males compared to controls, respectively, but differences were not considered biologically meaningful. Cause of death of early decedents seemed to show a trend in cause of male deaths due to pituitary tumors and female deaths due to mammary fibroadenoma with increasing drug treatment. However, there were no increases overall in pituitary or mammary tumors in relationship to drug treatment. See Sponsor's summary tables, below.

## Sponsor's mortality summary – Total Mortality

| | Group and sex | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1M | 2M | 3M | 4M | 1F | 2F | 3F | 4F |
| Dosage (mg/kg/day) | 0 | 6 | 18 | 60 | 0 | 6 | 18 | 60 |
| Group size | 55 | 55 | 55 | 55 | 55 | 55 | 55 | 55 |
| Total Number of deaths† | 17 | 17 | 20 | 21 | 21 | 21 | 16 | 23 |
| Number of survivors | 38 | 38 | 35 | 34 | 34 | 34 | 39 | 32 |
| % Survival | 69 | 69 | 64 | 62 | 62 | 62 | 71 | 58 |

† Includes animals found dead during the necropsy period

## Sponsor's mortality summary – Cause of Mortality

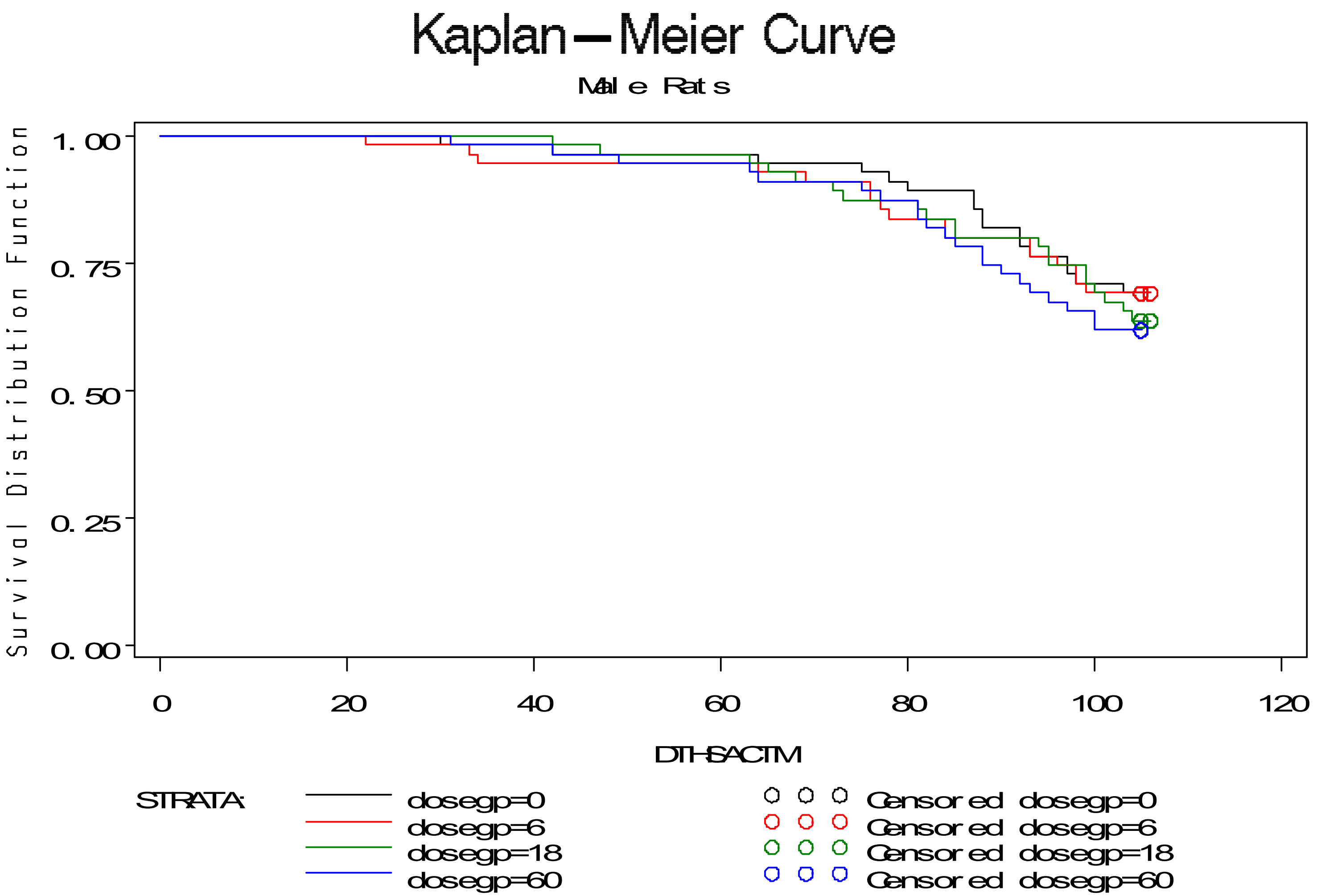
**Summary of major factors contributory to death in rats dying during the study**

| Group/sex | 1M | 2M | 3M | 4M | 1F | 2F | 3F | 4F |
|---|---|---|---|---|---|---|---|---|
| Dosage (mg/kg/day) | 0 | 6 | 18 | 60 | 0 | 6 | 18 | 60 |
| Pituitary tumours | | | | | | | | |
| Total | 4 | 6 | 14 | 12 | 14 | 11 | 9 | 11 |
| Mammary fibroadenoma | | | | | | | | |
| Total | 0 | 0 | 0 | 0 | 1 | 4 | 3 | 6 |
| Number of rats examined | 17 | 17 | 20 | 21 | 21 | 21 | 16 | 23 |

Kaplan-Meier survival plots from the FDA statistics review are shown below (M.A. Rahman, 2/21/11).

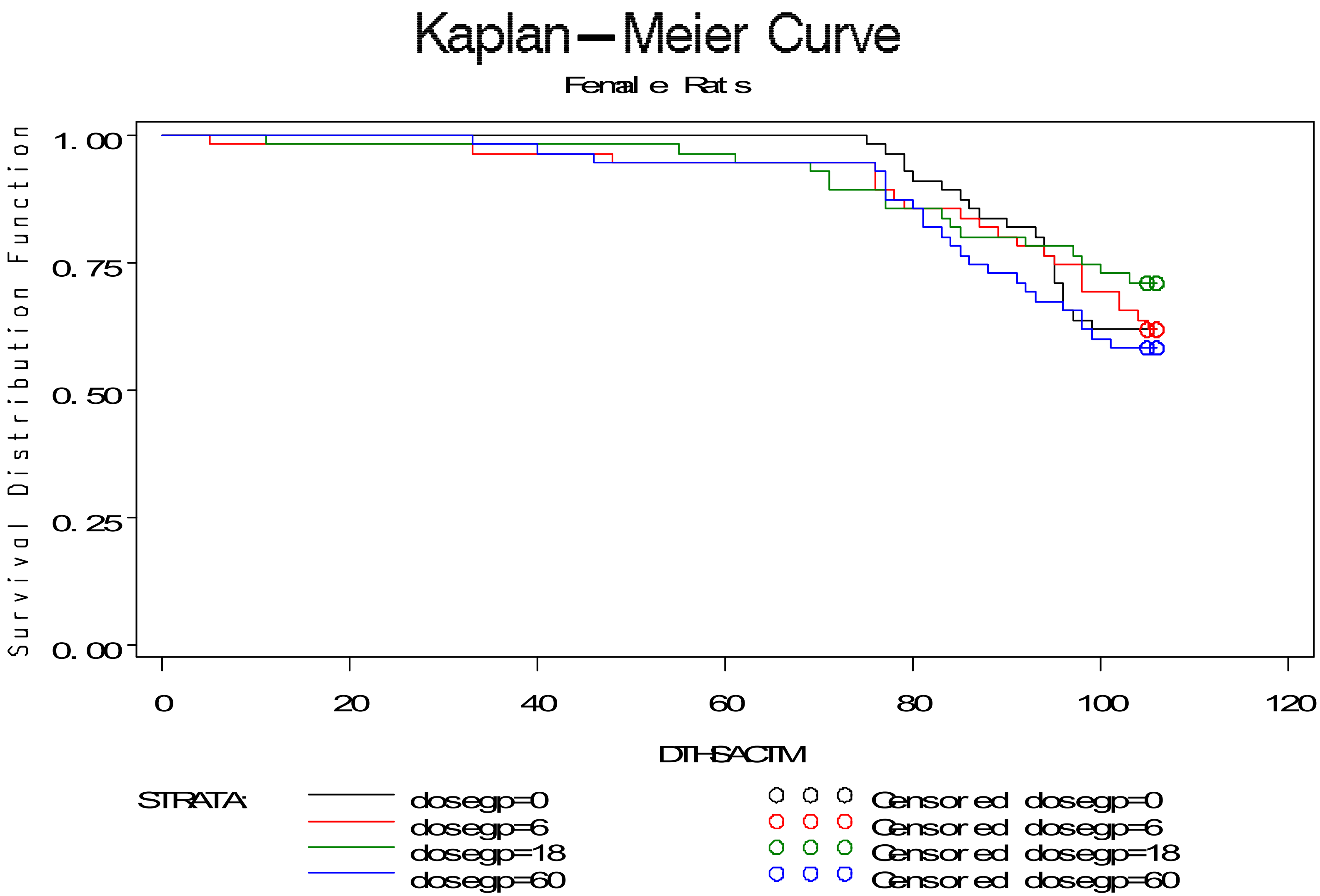
**Figure 8 – Kaplan Meier Survival Plots**

**Male Rats**




BEST POSSIBLE COPY

**Female Rats**




BEST POSSIBLE COPY

***Clinical Signs –*** Clinical signs were generally unremarkable. Salivation increased sporadically post-dose only in HD males and females over the first year of treatment. Salivation was transient and ceased within 1 h post-dose. Incidence in any week ranged from 0 to 45 animals, with only two weeks when salivation occurred in more than 10 animals in either males or females. Differences in weekly salivation observations may have been influenced by different reporting criteria for laboratory technicians. Nevertheless, there was no salivation in females after week 51 and only 7 observations in males were noted between weeks 52 and 63 and none after week 63.

***Body Weights –*** There were no remarkable body weight findings. Body weights (+4-7%, nss) and BW gain (+7=11%, nss) were slightly higher in treated female groups but there was no dose-related response.

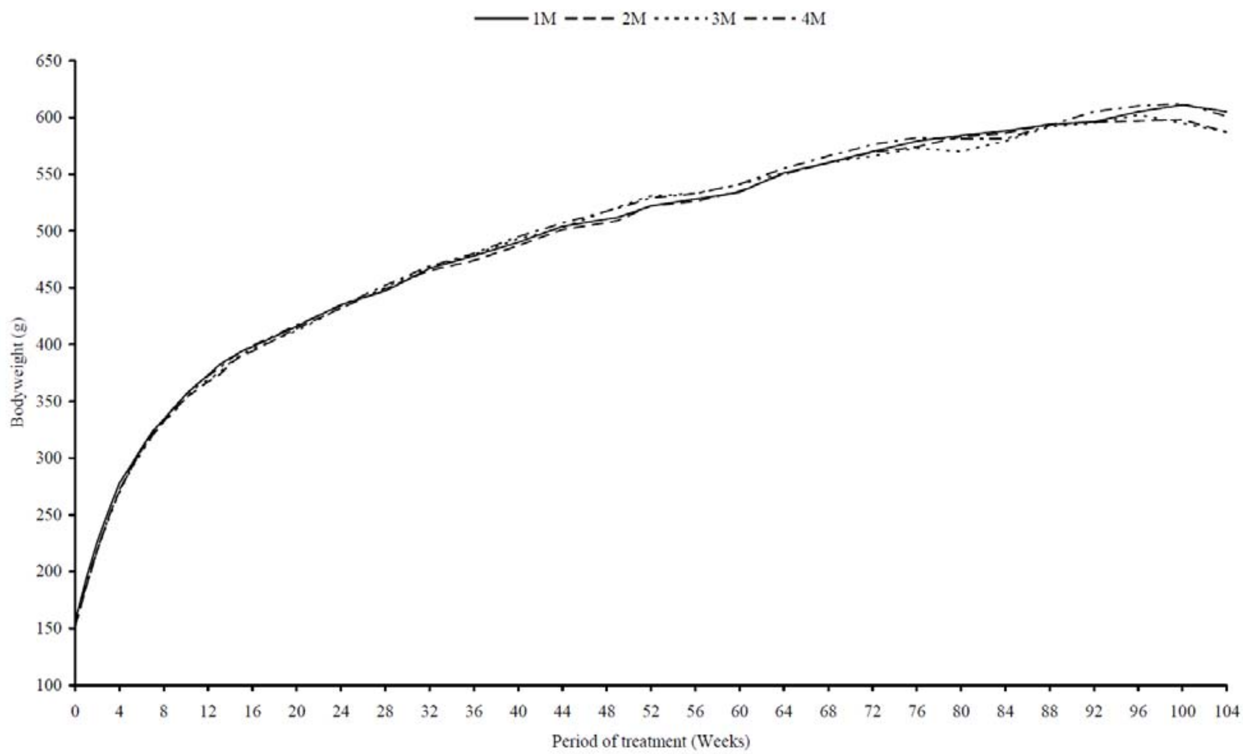
| Body Weight Summary | | | | |
|---|---|---|---|---|
| Treatment (mg/kg/day) | Male | | Female | |
| | BW (g) | BW Gain (g) | BW (g) | BW Gain (g) |
| 0 | 605 ± 60 | 448 ± 59 | 365 ± 48 | 240 ± 46 |
| 6 | 587 ± 72 | 437 ± 73 | 386 ± 53 | 262 ± 51 |
| 18 | 587 ± 83 | 435 ± 83 | 390 ± 57 | 267 ± 53 |
| 60 | 601 ± 73 | 448 ± 74 | 378 ± 50 | 256 ± 48 |

BW (body weight)
* p < 0.05, ** p < 0.01

**Figure 9 – Body Weight Curves**

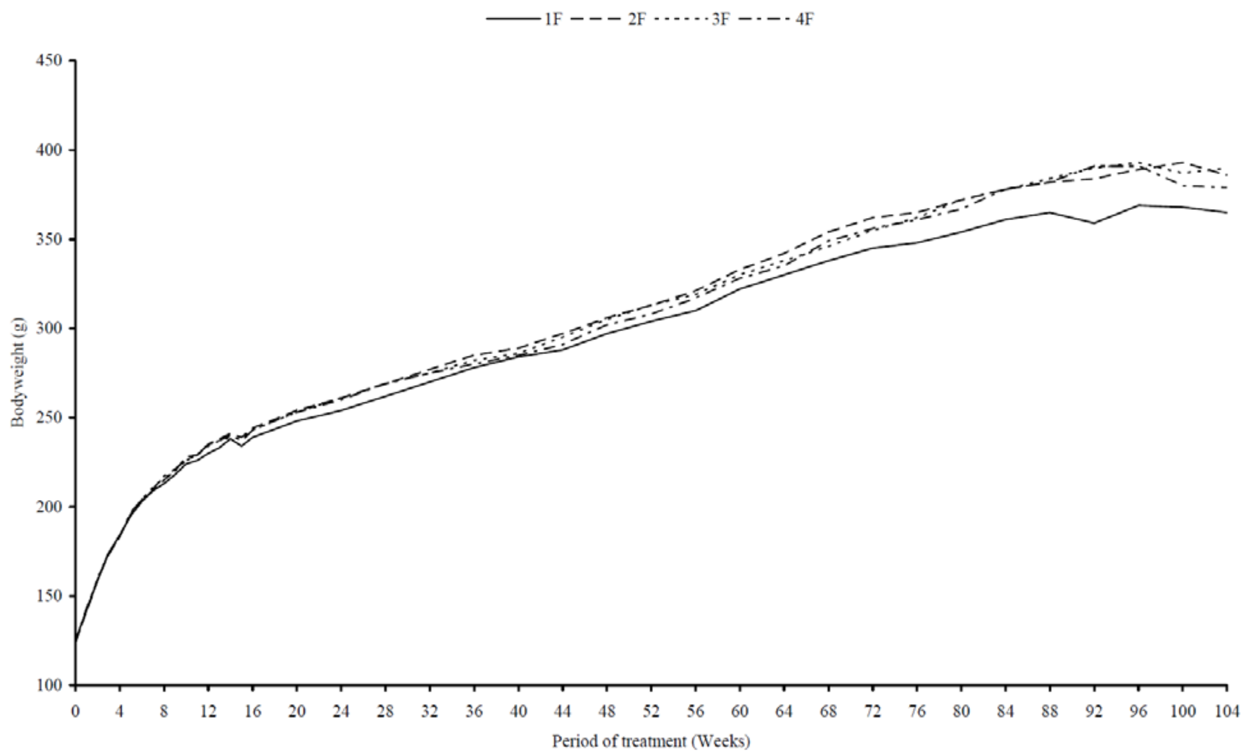
**Male**

| | | | | | |
|---|---|---|---|---|---|
| Group | : | 1 | 2 | 3 | 4 |
| Compound | : | Control | BI 1356 BS | BI 1356 BS | BI 1356 BS |
| Dosage (mg/kg/day) | : | 0 | 6 | 18 | 60 |




**Female**

| | | | | | |
|---|---|---|---|---|---|
| Group | : | 1 | 2 | 3 | 4 |
| Compound | : | Control | BI 1356 BS | BI 1356 BS | BI 1356 BS |
| Dosage (mg/kg/day) | : | 0 | 6 | 18 | 60 |




***Food Consumption –*** Unremarkable.

***Ophthalmology –*** Unremarkable.

***Clinical Pathology –*** Hematology findings were unremarkable. Various parameters were statistically different from concurrent controls but they were considered incidental, not dose-related, and/or not biologically significant. Certain findings are discussed here briefly or shown in the reviewer's summary table, below.

Total monocyte count increased slightly in male MD and HD (≤ 25%, nss) and females in all groups (+40-60%, not dose related). Platelet count was significantly increased in HD males (+12%, ss) and all female groups (+22%, +9%, +19%, respectively), but magnitude of increase was modest and there was no dose-relationship in females. Prothrombin time was slightly increased in LD females (+4%, ss) but not in other groups and, in contrast, APTT was decreased in MD (-18%, ss) and HD (-10%, ss) females. Absence of any clinical signs or effects on mortality further argued against any biologically significant treatment-related effects on blood clotting.

Clinical chemistry findings were similarly modest. Creatinine decreased modestly in MD (-21%, ss) and HD (-17%, ss) males but only slightly in HD (-3%, ss) females. Serum sodium was slightly decreased in MD (-2%) and HD (-2%) males and increased in HD (+1%, ss) females. There were no gross or histopathology findings correlated to the modest clinical chemistry findings. In addition, there was no plausible biological significance to slightly increased glucose in male LD (+28%, ss) and female MD (+19%) and HD (+12%), particularly since the overall pharmacodynamic effect is to decrease blood glucose post-dose. Various individual globulin protein concentrations (± 10-17%) and albumin:globulin ratio (+13%) were slightly altered in males, but there was no clear relationship to dose and findings were not considered biologically significant.

The Sponsor concluded there were no drug-related findings in animals sampled ante mortem prior to humane sacrifice throughout the study, which was consistent with the absence of any treatment related effect on mortality.

| Hematology and Clinical Chemistry Findings † | | | | | |
|---|---|---|---|---|---|
| **Parameter** | **Sex** | **BI 1356 BS (mg/kg/d)** | | | |
| | | **0** | **6** | **18** | **60** |
| Monocyte count ($10^9$/L) | M | 0.16 ± 0.06 | 0.16 ± 0.07 | 0.19 ± 0.07 (+19%) | 0.20 ± 0.07 (+25%) |
| | F | 0.10 ± 0.04 | 0.16 ±0.07** (+60%) | 0.14 ±0.05** (+40%) | 0.14 ±0.06** (+40%) |
| Platelet count ($10^9$/L) | M | 952 ± 155 | 928 ± 101 | 923 ± 140 | 1064 ± 190* (+12%) |
| | F | 793 ± 137 | 969±107** (+22%) | 865±129** (+9%) | 942±122** (+19%) |
| PT (s) | F | 14.6 ± 0.6 | 15.2 ±0.7* (+4%) | 14.7 ± 0.6 | 14.4 ± 0.6 |
| APTT (s) | F | 13.3 ± 2.1 | 13.0 ± 2.3 | 10.9± 2.9* (-18%) | 12.0 ±2.4* (-10%) |
| Creatinine (µM) | M | 42 ± 8 | 42 ± 4 | 33 ± 4** (-21%) | 35 ± 3** (-17%) |
| | F | 40 ± 3 | 40 ± 6 | 41 ± 7 | 39 ± 10* (-3%) |
| Na (mM) | M | 144± 1 | 145 ± 1 | 142 ± 1** (-2%) | 142 ± 1** (-2%) |
| | F | 140 ± 2 | 141 ± 1 | 141 ± 2 | 142 ± 1* (+1%) |

† Values ± SD and percent difference from concurrent control
* $p < 0.05$, ** $p < 0.01$ vs. vehicle control

***Urinalysis –*** Urinalysis findings were unremarkable. Total volume was decreased slightly in male HD (016%, ss) and increased in female HD (+42%, ss). Female control urine volume was low compared to all other treatment groups (16-42% lower) and compared to all male groups (33% lower than control males). Findings were considered unremarkable in the absence of any other changes in urine parameters or clinical chemistry markers (e.g., electrolyte concentrations).

***Gross Pathology –*** No apparent treatment-related findings in early decedents or at scheduled necropsy. There were no apparent drug-related increases in palpable masses.

***Histopathology***

***Peer Review –*** Multiple peer review was conducted, with a peer review conducted by the contract laboratory and independently by the Sponsor's pathologist.

***Neoplastic –*** There were no statistical increases in tumor incidence in either the Sponsor's analysis or in the independent FDA statistical analysis. See FDA statistics review for a list of combined tumors and a further discussion of tumor statistical analyses (M.A. Rahman, 2/21/11). Findings explained by the Sponsor or considered notable by this reviewer are discussed below.

Thyroid benign C-cell tumors seemed slightly elevated in male MD (24% incidence) and female LD (33% incidence) groups. Pair-wise comparisons were not statistically significant for these common tumors. There were no apparent increases in malignant C-cell tumors, but trends were similar for combined benign and malignant tumors as for benign tumors alone. Male (11%) and female (24%) concurrent control c-cell adenoma incidences were higher than usual, especially for females, compared to historical range in the conducting laboratory of 0-16% ($\bar{x}$ = 8.3%) and 2-24% ($\bar{x}$ = 8.7%) for males and females, respectively. C-cell findings are shown in the Sponsor's summary tables, below.

There were no clear treatment-related trends in C-cell hyperplasia, which is considered an earlier sign along a progression from hyperplasia to benign and malignant tumors. The absence of dose-related or statistical increases in benign tumors, absence of treatment-related increased C-cell hyperplasia, absence of increased malignant tumors, and an apparent increased background incidence in the study suggest c-cell adenomas were spontaneous and not treatment related.

Thyroid C-cell findings are further noteworthy because GLP-1 analogs listed for treatment of type 2 diabetes mellitus have caused increases in C-cell adenomas and carcinomas in rodents. DPP4 inhibitors act on the enzyme the breaks down the incretin hormone GLP-1, whereas GLP-1 analogs are synthetic peptides designed to be resistant to *in vivo* metabolism. Both DPP4 inhibitors and GLP-1 analogs presumably work by downstream effects of GLP-1 receptor activation. If the mechanism of thyroid C-cell tumor formation involves GLP-1 receptor mediated effects it is conceivable that DPP4 inhibitors could also cause increased C-cell tumors. Nevertheless, these data do not support a drug-related increase in C-cell adenomas and C-cell tumor induction is not listed on current labels of listed DPP4 inhibitors.

**Thyroid gland C-cell tumours**

| Group/Sex<br>Dosage (mg/kg/day) | | 1M<br>0 | 2M<br>6 | 3M<br>18 | 4M<br>60 | 1F<br>0 | 2F<br>6 | 3F<br>18 | 4F<br>60 |
|---|---|---|---|---|---|---|---|---|---|
| No. of animals examined | | 55 | 55 | 55 | 55 | 55 | 55 | 55 | 55 |
| C-cell adenomas | N | 6 | 9 | 13 | 8 | 13 | 18 | 16 | 11 |
| | % | 11 | 16 | 24 | 15 | 24 | 33 | 29 | 20 |
| C-cell carcinomas | N | 1 | 2 | 2 | 1 | 0 | 1 | 0 | 1 |
| | % | 2 | 4 | 4 | 2 | 0 | 2 | 0 | 2 |
| C-cell adenomas and carcinomas combined | N | 7 | 11 | 15 | 9 | 13 | 19 | 16 | 12 |
| | % | 13 | 20 | 27 | 16 | 24 | 35 | 29 | 22 |
| C-cell hyperplasia – diffuse | N | 24 | 32 | 17 | 29 | 30 | 37 | 30 | 33 |
| | % | 44 | 58 | 31 | 53 | 55 | 67 | 55 | 60 |
| C-cell hyperplasia – focal | N | 16 | 15 | 23 | 18 | 18 | 11 | 17 | 14 |
| | % | 29 | 27 | 42 | 33 | 33 | 20 | 31 | 25 |

## Historical Histopathology Data

### C-cell tumours

### Males

| Route of admin<br>Start date | dt<br>1/02 | dt<br>3/02 | dt<br>6/02 | dt<br>9/02 | ih<br>1/03 | ih<br>1/03 | og<br>3/03 | dt<br>6/03 | dt<br>1/04 | og<br>2/04 | og<br>7/04 | dt<br>1/05 | og<br>2/05 | og<br>3/06 | og<br>6/06 | Total | Range of % | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| c-cell adenoma | | | | | | | | | | | | | | | | | min | max |
| Incidence | 2 | 6 | 0 | 5 | 9 | 7 | 1 | 3 | 3 | 5 | 1 | 4 | 7 | 8 | 6 | 67 | | |
| Percentage % | 4.0 | 12.5 | 0.0 | 10.0 | 15.0 | 11.7 | 1.9 | 6.0 | 6.1 | 10.0 | 1.8 | 6.7 | 11.7 | 16.0 | 10.9 | 8.33 | 0.0 | 16.0 |
| c-cell carcinoma | | | | | | | | | | | | | | | | | | |
| Incidence | 1 | 0 | 2 | 0 | 4 | 0 | 1 | 1 | 0 | 0 | 1 | 3 | 0 | 1 | 1 | 15 | | |
| Percentage % | 2.0 | 0.0 | 3.6 | 0.0 | 6.7 | 0.0 | 1.9 | 2.0 | 0.0 | 0.0 | 1.8 | 5.0 | 0.0 | 2.0 | 1.8 | 1.87 | 0.0 | 6.7 |
| Number of animals examined | 50 | 48 | 55 | 50 | 60 | 60 | 52 | 50 | 49 | 50 | 55 | 60 | 60 | 50 | 55 | 804 | | |

### Females

| Route of admin<br>Start date | dt<br>1/02 | dt<br>3/02 | dt<br>6/02 | dt<br>9/02 | ih<br>1/03 | ih<br>1/03 | og<br>3/03 | dt<br>6/03 | dt<br>1/04 | og<br>2/04 | og<br>7/04 | dt<br>1/05 | og<br>2/05 | og<br>3/06 | og<br>6/06 | Total | Range of % | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| c-cell adenoma | | | | | | | | | | | | | | | | | min | max |
| Incidence | 2 | 3 | 2 | 6 | 7 | 5 | 5 | 1 | 1 | 4 | 4 | 5 | 9 | 3 | 13 | 70 | | |
| Percentage % | 4.0 | 6.1 | 3.6 | 12.0 | 11.7 | 8.5 | 9.6 | 2.0 | 2.0 | 8.2 | 7.3 | 8.3 | 15.3 | 6.0 | 23.6 | 8.72 | 2.0 | 23.6 |
| c-cell carcinoma | | | | | | | | | | | | | | | | | | |
| Incidence | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 1 | 2 | 0 | 0 | 10 | | |
| Percentage % | 2.0 | 0.0 | 1.8 | 2.0 | 1.7 | 1.7 | 0.0 | 0.0 | 4.0 | 0.0 | 0.0 | 1.7 | 3.4 | 0.0 | 0.0 | 1.25 | 0.0 | 4.0 |
| Number of animals examined | 50 | 49 | 55 | 50 | 60 | 59 | 52 | 50 | 50 | 49 | 55 | 60 | 59 | 50 | 55 | 803 | | |

Abbreviations

og - oral gavage
dt - dietary
ih - inhalation

Female mammary fibroadenoma was increased slightly in treatment groups, with 10, 18, 14, and 15 animals in respective groups. There was no significant dose-related treatment effect (nss, trend test) and none of the fibroadenoma incidence was statistically greater than controls (pair-wise).

The Sponsor discussed the biological significance of slightly increased skin fibroma in male treatment groups in the absence of any skin fibroma in control males. Fibroma incidence was highest in the LD group (7%), thus the trend test was not significant and neither was the pairwise comparison (p=0.06). The sponsor considered skin fibroma a ‘common’ tumor, although it did not reach statistical significance for either a ‘rare’ or ‘common’ tumor. The LD incidence was slightly outside the historical control incidence in the conducting lab (range 0-6%, mean not provided) and within the historical range of the more inclusive RITA database ($\overline{x}$ = 1.3%, range 0-8%, with no apparent breeder differences)[13]. The reviewing pathologist also noted three additional related mesenchymal tumors in control males, including skin benign fibrous histiocytoma and mammary fibroma. The reviewing pathologist considered the male skin fibromas in the context of all mesenchymal tumors, which further supported the absence of a treatment related increase in skin fibromas. This reviewer did not consider the male skin fibromas to be evidence of a drug-related tumor response based on the absence of a dose-related response, the low overall incidence near the historical control range, and the absence of a statistical increase compared to controls (even without considering benign tumors of similar mesenchymal origin).

**Mesenchymal tumours of the skin and mammary gland in male rats**

| Group/Sex<br>Dosage (mg/kg/day) | | 1M<br>0 | 2M<br>6 | 3M<br>18 | 4M<br>60 |
|---|---|---|---|---|---|
| No. of animals examined | | 55 | 55 | 55 | 55 |
| Skin fibroma | N | 0 | 4 | 1 | 2 |
| | % | 0 | 7 | 2 | 4 |
| Skin benign fibrous histiocytoma | N | 1 | 0 | 0 | 0 |
| | % | 2 | 0 | 0 | 0 |
| Skin lipoma | N | 1 | 0 | 0 | 0 |
| | % | 2 | 0 | 0 | 0 |
| Skin liposarcoma | N | 0 | 0 | 0 | 1 |
| | % | 0 | 0 | 0 | 2 |
| Mammary fibroma | N | 2 | 1 | 0 | 0 |
| | % | 4 | 2 | 0 | 0 |
| Mammary lipoma | N | 0 | 1 | 0 | 0 |
| | % | 0 | 2 | 0 | 0 |
| Skin and mammary gland mesenchymal tumours | N | 4 | 6 | 1 | 3 |
| | % | 7 | 11 | 2 | 5 |

[13] Morawietz G et al. (1992) RITA – Registry of Industrial Toxicology Animal Data. Progress of the working group. Exp Toxicol Pathol **44:**301-309

## Historical skin and mammary tumor data

**Skin and mammary tumours**

**Males**

| Route of admin<br>Start date | dt<br>1/02 | dt<br>3/02 | dt<br>7/02 | dt<br>9/02 | og<br>3/03 | dt<br>6/03 | dt<br>1/04 | og<br>2/04 | og<br>7/04 | dt<br>1/05 | Total | Range of % | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Mammary area** | | | | | | | | | | | | | |
| fibroma | | | | | | | | | | | | min | max |
| Incidence | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 2 | 5 | | |
| Percentage % | 0.0 | 0.0 | 0.0 | 2.0 | 1.9 | 0.0 | 0.0 | 2.0 | 0.0 | 3.3 | 0.96 | 0.0 | 3.3 |
| **Skin** | | | | | | | | | | | | | |
| fibroma | | | | | | | | | | | | | |
| Incidence | 3 | 1 | 0 | 1 | 0 | 1 | 2 | 0 | 1 | 1 | 10 | | |
| Percentage % | 6.0 | 2.0 | 0.0 | 2.0 | 0.0 | 2.0 | 4.0 | 0.0 | 1.8 | 1.7 | 1.92 | 0.0 | 6.0 |
| lipoma | | | | | | | | | | | | | |
| Incidence | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | | |
| Percentage % | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.19 | 0.0 | 2.0 |
| liposarcoma | | | | | | | | | | | | | |
| Incidence | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | | |
| Percentage % | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.0 | 0.0 | 0.0 | 0.19 | 0.0 | 2.0 |
| Number of animals examined | 50 | 50 | 55 | 50 | 52 | 50 | 50 | 50 | 55 | 60 | 522 | | |

Abbreviations

og - oral gavage
dt - dietary
ih - inhalation

### *Non-Neoplastic*

Non-neoplastic findings were limited. Lung cholesterol cleft granuloma(ta) incidence increased in male and female HD groups. Overall cholesterol cleft incidence were modest in HD males (15/55, 27%) and females (15/55, 27%), but trends in both sexes suggest a drug-related increase compared to control males (6/55, 11%) and females (7/55, 13%). The study pathologist noted granuloma(ta) morphology was similar across treatment groups, including controls, arguing against a tissue-specific drug-related effect. This reviewer notes that lesions suggesting drug-related lung phospholipidosis (e.g., foamy cell macrophages) were seen in shorter duration rat studies. Cholesterol cleft granuloma(ta) are consistent with the presence of macrophages in other studies. There were no other apparent neoplastic or non-neoplastic drug-related effects in lung. This reviewer considered the cholesterol cleft granulomas(tas) drug-related in the HD groups, however, based on the absence of a significant toxicological correlate (e.g., increased clinical signs, mortality, or evidence of progression to neoplasms), the biological significance of chronic lung phospholipid findings are unclear.

**Cholesterol cleft granuloma(ta) of the lungs**

| Group/Sex<br>Dosage (mg/kg/day) | | 1M<br>0 | 2M<br>6 | 3M<br>18 | 4M<br>60 | 1F<br>0 | 2F<br>6 | 3F<br>18 | 4F<br>60 |
|---|---|---|---|---|---|---|---|---|---|
| No. of animals examined | | 55 | 55 | 55 | 55 | 55 | 55 | 55 | 55 |
| Cholesterol cleft granuloma(ta) | N | 6 | 6 | 8 | 15 | 7 | 4 | 6 | 15 |
| | % | 11 | 11 | 15 | 27 | 13 | 7 | 11 | 27 |

Additional non-neoplastic findings considered noteworthy are shown below (from various Sponsor's tables). None of the findings were considered clearly drug-related or biologically significant, based on absence of clear dose-response or correlative toxicological endpoints. There were no drug-related effects on kidney tumors, but metastatic transitional epithelial mineralization and reactive transitional epithelial hyperplasia were increased in most treatment groups. Mineralization incidence was increased in HD males (32/55 vs. 21/55 controls), but severity was limited to minimal or slightly mineralization in all but 1/55 HD males. There was no clear increase in severity of kidney mineralization or hyperplasia in other treatment groups. The biological significance and potential drug-related effect of kidney lesions is questionable based on the high background incidence in controls and absence of either a tumor response, an effect on chronic kidney function (e.g., chronic progressive nephropathy), or contribution to early deaths after chronic exposure.

Thyroid cystic follicular cell hyperplasia seemed slightly increased in MD and HD males and females, but overall incidence was low and biological significance is not clear.

Epididymid perivascular inflammatory cells (7, 10, 11, 16, respectively) seemed slightly increased in treated males. Lesions were typically focal in nature, generally seen only in aged rats at terminal necropsy, and generally limited to minimal severity with no increased severity with increased dose. The slight increase in incidence in aged males was not considered biologically significant by this reviewer.

**Metastatic transitional epithelial mineralization and reactive transitional epithelial hyperplasia of the kidney**

| Group/Sex<br>Dosage (mg/kg/day) | | 1M<br>0 | 2M<br>6 | 3M<br>18 | 4M<br>60 | 1F<br>0 | 2F<br>6 | 3F<br>18 | 4F<br>60 |
|---|---|---|---|---|---|---|---|---|---|
| No. of animals examined | | 55 | 55 | 55 | 55 | 55 | 55 | 55 | 55 |
| Transitional epithelial mineralization | N | 21 | 20 | 27 | 32 | 45 | 52 | 51 | 51 |
| | % | 38 | 36 | 49 | 58 | 82 | 95 | 93 | 93 |
| Transitional epithelial hyperplasia | N | 14 | 18 | 16 | 21 | 36 | 50 | 41 | 46 |
| | % | 25 | 33 | 29 | 38 | 65 | 91 | 75 | 84 |

Histopathology - group distribution of non-neoplastic findings for all animals

| Group | : | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| Compound | : | Control | BI 1356 BS | BI 1356 BS | BI 1356 BS |
| Dosage (mg/kg/day) | : | 0 | 6 | 18 | 60 |

| Tissue and Finding | Group/Sex: Number: | 1M 55 | 2M 55 | 3M 55 | 4M 55 | 1F 55 | 2F 55 | 3F 55 | 4F 55 |
|---|---|---|---|---|---|---|---|---|---|
| Kidneys | Number Examined: | 55 | 55 | 55 | 55 | 55 | 55 | 55 | 55 |
| Pelvic/Transitional Epithelial Mineralisation | | 21 | 20 | 27 | 32 | 45 | 52 | 51 | 51 |
| Pyelitis | | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Transitional Epithelial Hyperplasia | | 14 | 18 | 16 | 21 | 36 | 50 | 41 | 46 |
| Pancreas | Number Examined: | 55 | 55 | 55 | 55 | 55 | 55 | 55 | 55 |
| Acinar Atrophy, Focal | | 15 | 10 | 15 | 12 | 7 | 13 | 13 | 13 |
| Thyroids | Number Examined: | 55 | 55 | 55 | 55 | 55 | 55 | 55 | 55 |
| Cystic Follicular Cell Hyperplasia | | 6 | 3 | 8 | 11 | 0 | 0 | 2 | 5 |
| Diffuse C-Cell Hyperplasia | | 24 | 32 | 17 | 29 | 30 | 37 | 30 | 33 |
| Ectopic Thymic Tissue | | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Focal C-Cell Hyperplasia | | 16 | 15 | 23 | 18 | 18 | 11 | 17 | 14 |
| Follicular Cell Hypertrophy | | 2 | 3 | 0 | 0 | 0 | 1 | 1 | 1 |
| Pituitary | Number Examined: | 55 | 53 | 55 | 55 | 54 | 55 | 55 | 54 |
| Hyperplasia - Pars Distalis, Focal | | 1 | 4 | 3 | 2 | 8 | 5 | 8 | 11 |
| Hyperplasia - Pars Intermedia, Focal | | 1 | 2 | 0 | 1 | 2 | 0 | 0 | 0 |
| Hypertrophy - Pars Distalis, Focal | | 2 | 3 | 5 | 3 | 2 | 3 | 0 | 3 |
| Epididymides | Number Examined: | 55 | 55 | 55 | 55 | - | - | - | - |
| Perivascular Inflammatory Cells | | 7 | 10 | 11 | 16 | - | - | - | - |

***Toxicokinetics –*** Drug was measured in treated groups with exposure increased greater than dose proportionally. Variability within groups ranged from 16% to 80% for BI 1356 BS (coefficient of variation at $C_{max}$) and was higher for metabolite CD 1750 XX. Exposure increased markedly after repeated exposure, with up to approximately 3- to 11-fold higher exposure ($C_{max}$ and AUC) on day 179 compared to day 1. Drug levels did achieve steady state levels, with plasma drug and metabolite concentrations on day 361 similar to those on day 179. There was no consistent sex difference in exposure. CD 1750 XX exposure was approximately 6-8% of parent. Mean exposure data are shown in the Sponsor's summary tables, below.

BI 1356 BS (but not metabolite CD 1750 XX) was detected in 2 of 226 control samples. The BI 1356 BS concentrations were 4.5-7.7 nmol/l, which were below LD levels at the respective time points. Contamination origin was not determined but the drug was only detected infrequently, at low levels, and in the absence of metabolite. Conditions suggest potential contamination post-blood sampling, regardless, the study results are considered valid and study integrity did not seem to be compromised by the finding in control blood samples.

**Linagliptin TK Summary**

| Parameter | Day | Gender | 6 mg/kg | 18 mg/kg | 60 mg/kg |
|---|---|---|---|---|---|
| C(max) [nmol/L] | 1 | m | 24.7 | 187 | 2640 |
| | 1 | f | 35.5 | 414 | 2890 |
| | 179 | m | 273 | 1430 | 7090 |
| | 179 | f | 105 | 1130 | 6230 |
| | 361 | m | 201 | 1380 | 7920 |
| | 361 | f | 272 | 1150 | 6040 |
| AUC(0-24h) [nmol·h/L] | 1 | m | 314 | 1170 | 15900 |
| | 1 | f | 296 | 1760 | 17900 |
| | 179 | m | 1600 | 8570 | 62000 |
| | 179 | f | 989 | 5540 | 48900 |
| | 361 | m | 1550 | 9930 | 70100 |
| | 361 | f | 1480 | 6220 | 62000 |

**CD 1750 XX (Metabolite) TK Summary**

| Parameter | Day | Gender | 6 mg/kg | 18 mg/kg | 60 mg/kg |
|---|---|---|---|---|---|
| C(max) [nmol/L] | 1 | m | 0.212 | 5.75 | 114 |
| | 1 | f | 1.47 | 18.6 | 98.8 |
| | 179 | m | 13.2 | 114 | 477 |
| | 179 | f | 10.7 | 107 | 379 |
| | 361 | m | 10.1 | 89.3 | 413 |
| | 361 | f | 27.2 | 108 | 367 |
| AUC(0-24h) [nmol·h/L] | 1 | m | 0.636 | 27.4 | 747 |
| | 1 | f | 6.46 | 73.3 | 701 |
| | 179 | m | 41.9 | 521 | 4470 |
| | 179 | f | 70.8 | 467 | 3010 |
| | 361 | m | 53.4 | 523 | 4030 |
| | 361 | f | 121 | 485 | 3510 |

***Stability and Homogeneity –*** Stock assay solutions were tested appropriately for stability, homogeneity, and drug concentration. Mean concentrations ranged from 89.4% to 102% of nominal concentrations. Various individual samples were outside the ± 10% nominal range but deviations were infrequent and did not affect the integrity of the study over the 2-year dosing period.

## Mouse 2 year oral carcinogenicity study

*0, 8, 25, 80 mg/kg/d (oral gavage)*
*0.8, 5, 38 µM*h (5X, 32X, 241X MRHD)*

**Key Study Findings:**

*NOAEL (neoplastic) = Males: 80 mg/kg (271X MRHD estimate)*
*Females: 25 mg/kg (34X MRHD estimate)*

*NOAEL (non-neoplastic) = Females: 80 mg/kg (215X MRHD estimate)*
*Males: 25 mg/kg (31X MRHD estimate)*

Adequacy of Carcinogenicity Study – The final study report of a GLP-compliant, standard two year oral (gavage) carcinogenicity study in CD-1 mouse was reviewed and results were discussed at a meeting of the Executive Carcinogenicity Assessment Committee (ECAC). The study was considered acceptable based on doses previously recommended by the ECAC and which provide exposure greater than 25-times the expected maximum human dose. Satellite animals were included for toxicokinetic analyses and mouse exposures could be compared to expected human exposure.

Appropriateness of Test Models – The Sponsor chose doses of 0, 8, 25, and 80 mg/kg/day linagliptin (BI 1356 BS) based on previous recommendations of the ECAC. Treatment was well tolerated and results showed no dose-limiting toxicity up to the highest dose tested. Mean exposures, sexes combined, were approximately 5X, 32X, and 241X the expected human exposure (MRHD) based on total exposure ($AUC_{0-24}$).

Evaluation of Tumor Findings – Statistically significant increased tumors were limited to malignant lymphoma in females treated with the high dose of 80 mg/kg/d. Concurrent control lymphoma incidence was approximately 40% below the low end of the conducting laboratory's historical control range, potentially confounding interpretation of the HD tumor findings. Nevertheless, lymphoma in the high dose was also clearly higher than other treatment groups, outside the conducting lab's historical control range, and highly statistically significant for both dose-response trend and pair-wise analyses. No other tumors in mice were statistically significant or considered biologically significant or treatment related. Drug-related increased malignant lymphoma occurred in only one sex at approximately 287-times the MRHD based on total female exposure ($AUC_{0-24}$). Using a NOAEL of 25 mg/kg/d (MD) for females and 80 mg/kg/d (HD) for males, mouse exposures that did not result in any drug-induced tumors were approximately 34-fold (females) and 271-fold (males) higher than maximum expected human exposures.

Evaluation of Non-Neoplastic Findings – Non-neoplastic findings were largely unremarkable and treatment did not affect survival. There was an increase in grossly enlarged mandibular lymph nodes in HD females, which was consistent with increased lymphomas. Gross findings in males included increased lung masses in all groups and dose-related increases in testes 'prominent tubules'. Male reproductive tissue lesions were also seen histologically, including increased severity of germ cell depletion, increased incidence and severity of testes mineralization, and epididymid increased duct dilatation, slightly increased epithelial dilatation and decreased numbers of spermatozoa. The relationship between gross and histologic testes findings was not clear but they may be related. The male testes findings were considered adverse based on generally increased incidence and severity in the high dose group. It is not clear whether male testes findings would affect fertility and it is notable that drug-related findings occurred in aged mice. Male fertility was not affected in a separate dedicated rat fertility study.

**Title – BI 1356 BS: Carcinogenicity study by oral gavage administration to CD-1 mice for 104 weeks**

| | |
|---|---|
| Study no.: | BOI0330 (Doc. No. U10-1500-01) |
| Study report location: | eCTD 4.2.3.4.1 |
| Conducting laboratory and location: | [redacted] (b) (4) |
| Date of study initiation: | 6/26/06 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | BI 1356 BS, Batch No. 5060170, 97.7-97.9% purity |
| CAC concurrence: | Yes |

## Methods

| | |
|---|---|
| Doses: | 0, 8, 25, 80 mg/kg/d |
| Frequency of dosing: | QD |
| Dose volume: | 10 ml/kg |
| Route of administration: | Oral (gavage) |
| Formulation/Vehicle: | 0.5% aqueous hydroxyethylcellulose (Natrosol® 250 HX) |
| Basis of dose selection: | > 25X AUC at MRHD |
| Species/Strain: | CD-1 mouse |
| Number/Sex/Group: | 60 |
| Age: | 5-6 weeks |
| Animal housing: | 3 ♀/group (♂ housed individually) |
| Paradigm for dietary restriction: | None (*ad lib.* feeding) |
| Dual control employed: | No |
| Interim sacrifice: | No |
| Satellite groups: | 18/sex controls, 35/sex/treatment group |
| Deviation from study protocol: | |

**Study Design Summary**

| Group | Treatment | Dosage# (mg/kg/day) | Main study No. of animals Male | Female | Main study Animal numbers Male | Female | Satellite study Animal numbers Male | Female |
|---|---|---|---|---|---|---|---|---|
| 1 | Control | 0 | 60 | 60 | 1-60 | 364-423 | 61-78 | 424-441 |
| 2 | BI 1356 BS | 8 | 60 | 60 | 79-138 | 442-501 | 139-173 | 502-536 |
| 3 | BI 1356 BS | 25 | 60 | 60 | 174-233 | 537-596 | 234-268 | 597-631 |
| 4 | BI 1356 BS | 80 | 60 | 60 | 269-328 | 632-691 | 329-363 | 692-726 |

\# Expressed in terms of test material as supplied.

Observations and Results

***Statistics –*** Statistical analyses were conducted by the Sponsor and independently by the FDA. Statistical trend test for dose response and pair-wise test for differences between individual treatment groups and controls were conducted for mortality and tumor incidence. The Sponsor followed international guidance for tumor analyses, considering “common” tumors (> 1% historical incidence) significant at $p < 0.005$ and $p < 0.01$ and “rare” tumors (< 1% historical incidence) significant at $p < 0.025$ and $p < 0.05$, for trend and pair-wise tests, respectively.

The Sponsor considered the following tumors to be “rare” in their analysis (with all others considered “common”):

**Males**

Kidneys - Malignant tubular carcinoma
Kidneys - Benign tubular adenoma and malignant tubular carcinoma combined
Spleen - Malignant haemangiosarcoma
Pancreas - Benign Islet cell adenoma
Mesenteric lymph node - Benign haemangioma
Skeletal muscle - Malignant haemangiosarcoma
Duodenum - Benign adenoma and malignant adenocarcinoma combined
Haematopoietic tumour - Malignant myeloid cell leukaemia

**Females**

Pancreas - Benign Islet cell adenoma
Skin - Benign trichoepithelioma
Ovaries - Benign sertoliform tubular adenoma
Uterus - Malignant leiomyosarcoma
Uterine cervix - Benign endometrial polyp

***Mortality –*** Survival in mice was 50%, 50%, 58%, 68% in males and 47%, 35%, 47%, and 33% in females in respective groups (see Sponsor’s table, below). Treatment did not significantly increase mortality for males or females. The Sponsor’s analysis showed a dose-related increase in survival for males, while an independent FDA analysis showed no significant trend but a significant increase in male survival in the HD by pairwise comparison. Statistical findings were similar in the Sponsor's and FDA's analyses, however, the FDA reviewer considered animals that died naturally during the final study week 104 to be "survivors" while the Sponsor only counted live animals at scheduled necropsy as survivors. Overall, sufficient numbers of animals survived until terminal necropsy for carcinogenicity assessment and there was no apparent increased mortality with BI 1356 BS treatment.

There were no data to clearly support an explanation for increased male survival. Body weights were decreased slightly in HD males (-3%, nss), which was not likely to have a biologically significant effect on survival. There was no difference in chronic progressive nephropathy across treatment groups.

| | Group and sex | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **1M** | **2M** | **3M** | **4M** | **1F** | **2F** | **3F** | **4F** |
| **Dosage (mg/kg/day)** | **0** | **8** | **25** | **80** | **0** | **8** | **25** | **80** |
| Group size | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| Total Number of deaths† | 31 | 30 | 25 | 21 | 32 | 40 | 32 | 41 |
| Number of survivors | 29 | 30 | 35 | 39 | 28 | 20 | 28 | 19 |
| % Survival | 48 | 50 | 58 | 65 | 47 | 33 | 47 | 32 |

† Includes animals found dead during the necropsy period

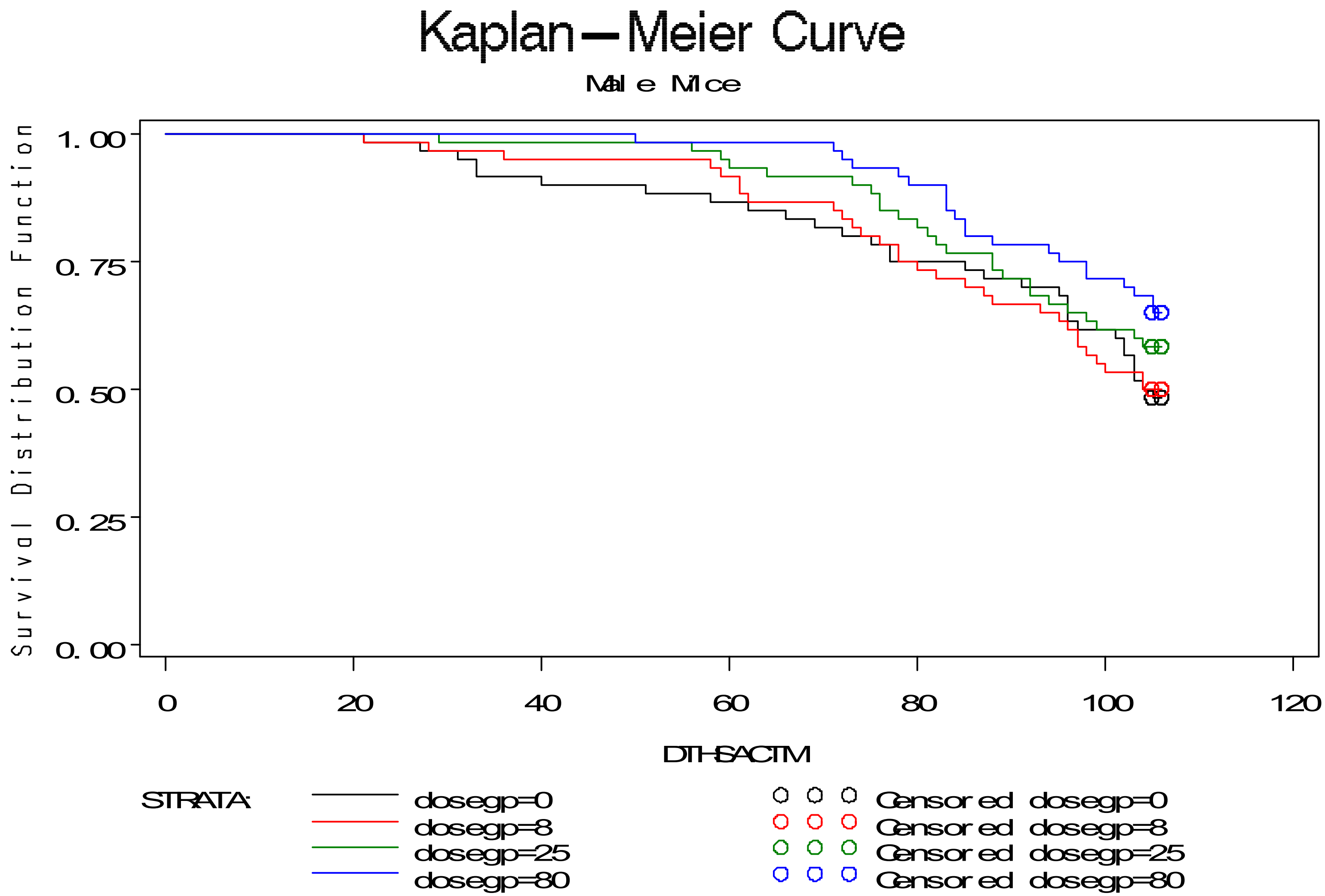
Lymphoma was the most common cause of early death across most treatment groups, including a very high incidence (18 deaths) in female HD but no cases in male HD. The study pathologist considered the most common causes of death to be within the normal range of findings in the mouse strain (see Sponsor's table, below).

**Summary of major factors contributory to death in mice dying during the study**

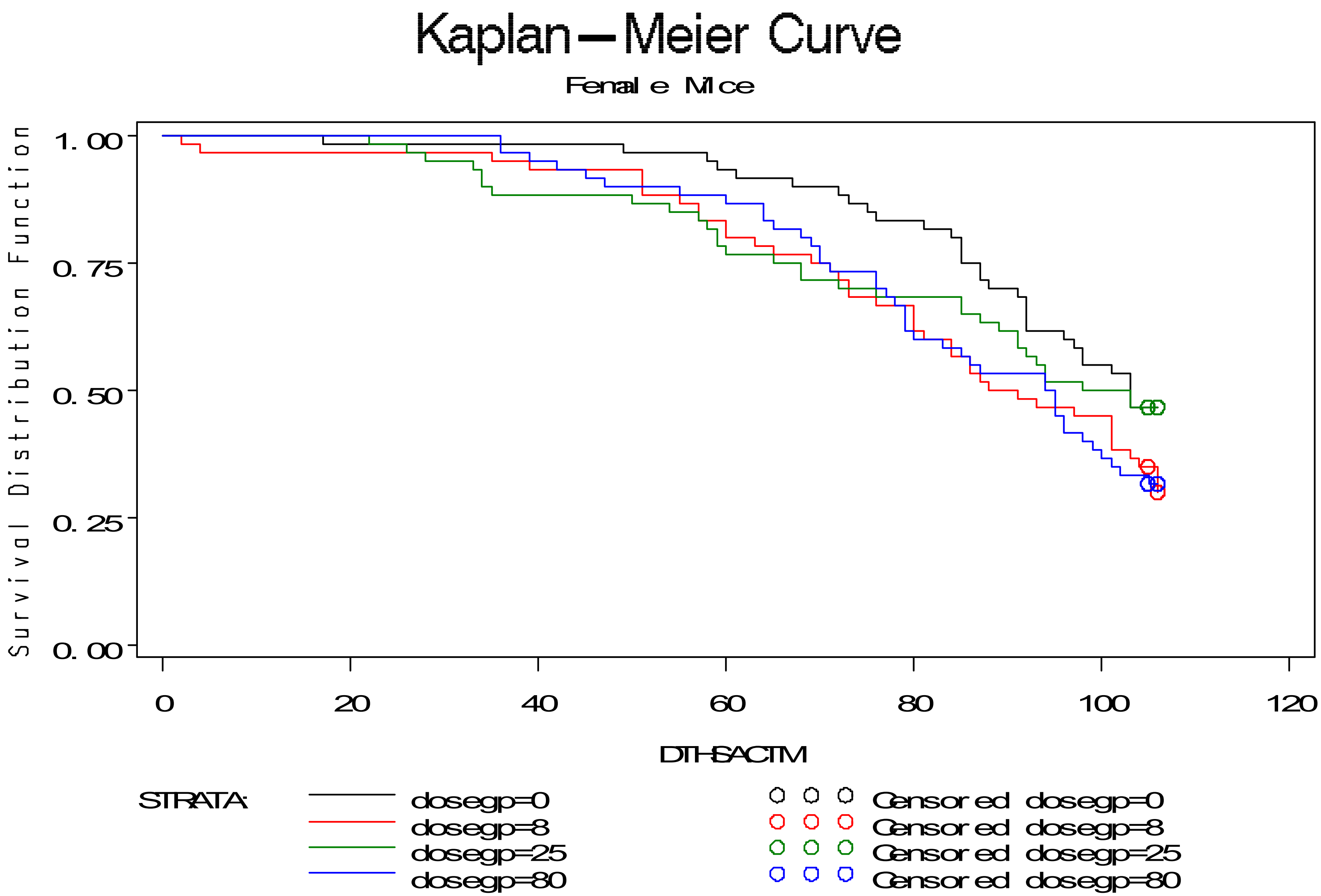
| **Group/sex** | | **1M** | **2M** | **3M** | **4M** | **1F** | **2F** | **3F** | **4F** |
|---|---|---|---|---|---|---|---|---|---|
| **Dosage (mg/kg/day)** | | **0** | **8** | **25** | **80** | **0** | **8** | **25** | **80** |
| Lymphoma | Total | 8 | 4 | 4 | 0 | 5 | 10 | 7 | 18 |
| Bronchioloalveolar adenocarcinoma | Total | 3 | 6 | 3 | 5 | 3 | 3 | 2 | 1 |
| Kidney lesions | Total | 2 | 1 | 0 | 0 | 3 | 3 | 4 | 7 |
| Urogenital lesions | Total | 4 | 2 | 4 | 3 | 1 | 0 | 2 | 1 |
| Skin/subcutis lesions | Total | 2 | 1 | 2 | 1 | 5 | 4 | 3 | 0 |
| Number of mice examined | | 31 | 30 | 25 | 21 | 32 | 40 | 32 | 41 |

Kaplan-Meier survival plots from the FDA statistics review are shown below (M.A. Rahman, 2/21/11).

**Figure 10 – Kaplan Meier Survival Plots**




BEST POSSIBLE COPY




BEST POSSIBLE COPY

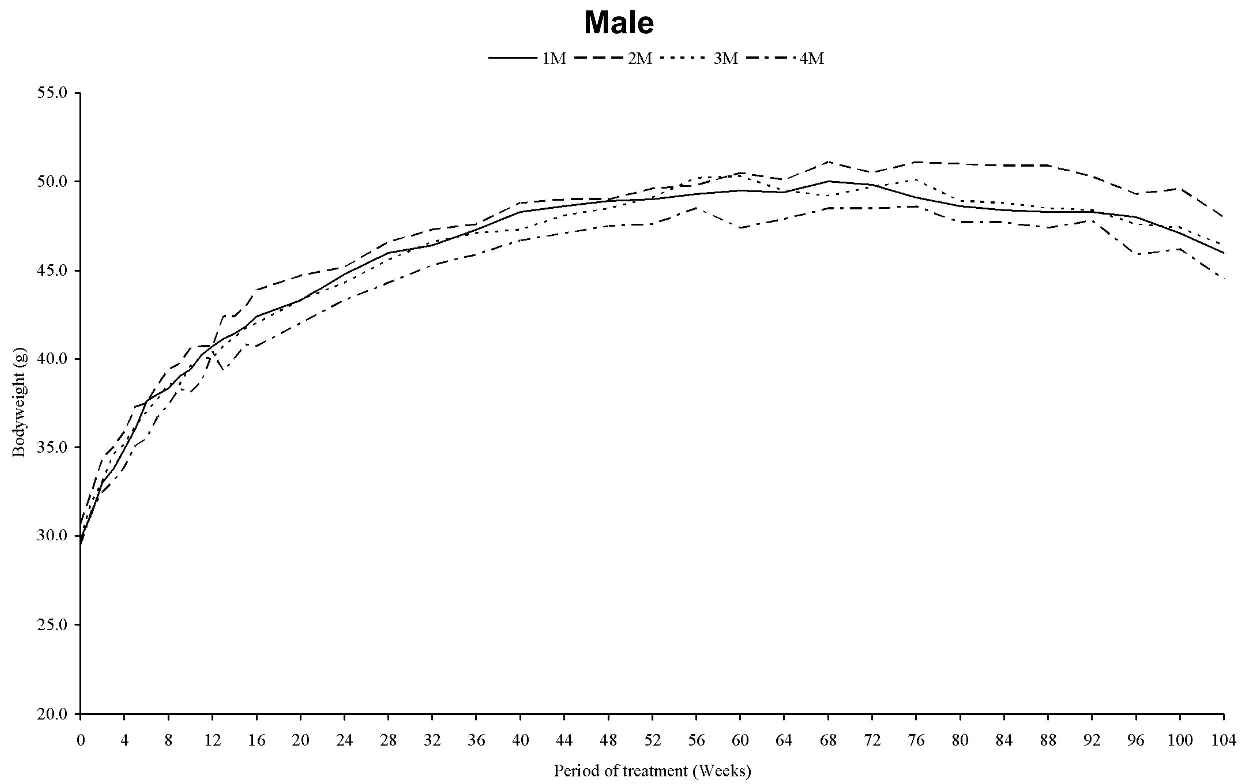
***Clinical Signs –*** Unremarkable. Signs were considered consistent with findings in the strain, particularly with signs of aging as the chronic study progressed.

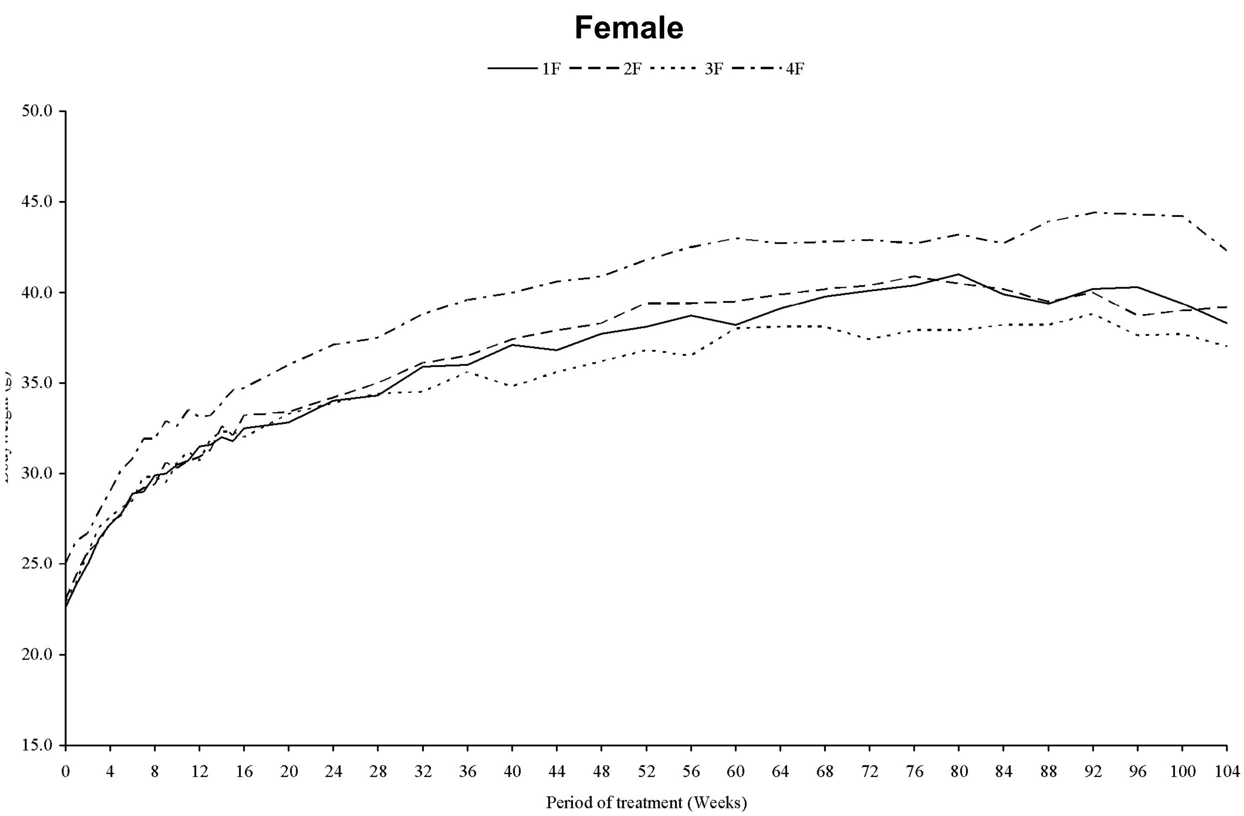
***Body Weights –*** There were no clear treatment-related effects on body weight. There were some slight differences between control and various treatment groups, but there were no dose-related trends and all differences were modest (± 10%). Summary BW data are shown in the reviewer's table and Sponsor's figures, below.

| Body Weight Summary | | | | |
|---|---|---|---|---|
| **Treatment (mg/kg/day)** | **Male** | | **Female** | |
| | **BW (g)** | **BW Gain (g)** | **BW (g)** | **BW Gain (g)** |
| 0 | 46.0 ± 4.7 | 15.8 ± 4.5 | 38.3 ± 5.7 | 15.8 ± 5.1 |
| 8 | 48.0 ± 5.2 | 17.0 ± 4.9 | 39.2 ± 4.8 | 15.9 ± 4.1 |
| 25 | 46.4 ± 6.7 | 16.7 ± 6.2 | 37.0 ± 4.8 | 14.2 ± 4.1 |
| 80 | 44.5 ± 5.5 | 15.2 ± 4.7 | 42.3 ± 7.4 | 17.6 ± 6.7 |

BW (body weight)

**Figure 11 – Body Weight Curves**



***Food Consumption –*** Food consumption was slightly higher in female treatment groups compared to controls. Differences were not significant throughout the study, however, the trend was consistent and mean consumption over the course of the study was increased 9%, 12%, and 15% in respective treatment groups compared to controls. Increased food consumption was correlated with 10% increased body weight in HD females compared to controls, but not with any other female BW trends. There were no apparent effects on food consumption in males.

***Clinical Pathology –*** Findings were unremarkable. There were sporadic significant differences in individual parameters compared to controls, but findings were not dose related and not considered biologically significant. Mean cell hemoglobin was increased slightly (5-6%) in all female treatment groups, but there was no dose-related trend.

***Gross Pathology –*** There were no treatment-related effects on the overall number of palpable masses (see Sponsor's summary table, below). There were differences between treatment groups and controls for various individual masses or other gross lesions (reviewer's discussion and summary table, below). The biological significance of treatment-related gross lesions is not clear, particularly in the absence of any drug-related increase in mortality over the course of chronic treatment.

### Palpable mass summary

Palpable swellings - group distribution, multiplicity and mean time of onset+

| | | | | | |
|---|---|---|---|---|---|
| Group | : | 1 | 2 | 3 | 4 |
| Compound | : | Control | BI 1356 BS | BI 1356 BS | BI 1356 BS |
| Dosage (mg/kg/day) | : | 0 | 8 | 25 | 80 |

| Group /Sex | Multiplicity∅ 0 | 1 | 2 | 3 | 4 or more | Number of animals with swellings | Total number of swellings | Mean time of onset* |
|---|---|---|---|---|---|---|---|---|
| 1M | 43 | 12 | 1 | 3 | 1 | 17 | 28 | 57 |
| 2M | 48 | 10 | 2 | 0 | 0 | 12 | 14 | 65 |
| 3M | 51 | 6 | 3 | 0 | 0 | 9 | 12 | 66 |
| 4M | 46 | 8 | 5 | 1 | 0 | 14 | 21 | 73 |
| 1F | 51 | 7 | 1 | 0 | 1 | 9 | 14 | 61 |
| 2F | 52 | 5 | 1 | 2 | 0 | 8 | 13 | 59 |
| 3F | 53 | 5 | 2 | 0 | 0 | 7 | 9 | 72 |
| 4F | 50 | 8 | 1 | 1 | 0 | 10 | 13 | 56 |

\+ Including swellings which regressed or were not positively identified at *post mortem* examination
∅ Expressed as number of animals bearing the indicated number of swellings
* In weeks to onset of first recorded swelling including those found at necropsy examination

Gross examination showed increased lung masses in treated male groups, with no relationship to dose. The biological significance was not clear. Gross lung masses may be correlated with a trend (nss) of increased bronchioloalveolar adenoma and carcinoma (individual and combined) in males. Bronchioloalveolar tumor incidence, combined, was 16, 24, 29, and 24 in respective male groups (compared to 20, 30, 29, and 26 gross lung masses).

Incidence of enlarged mandibular lymph nodes was increased in female HD compared to controls (7, 10, 8, 14 in respective female groups). Gross masses were correlated with increased lymphomas in female groups, particularly HD.

There was a dose-related increase in adrenal masses in males, with 5, 8, 9, and 12 in respective male groups. Tumors observed at necropsy were actually lower than total masses noted grossly but there were high incidences of adrenal cortical hypertrophy (22, 8, 9, 28, respectively) and a few cases of adrenal cortical focal hyperplasia (1, 4, 2, 0, respectively). Adrenal masses likely identified some cases of adrenal hypertrophy in addition to benign tumors.

There was also a dose-related increase in prominent tubules in the testes of treated males, with 4, 13, 17, and 18 in respective male groups. Other testes and male reproductive tissue findings were seen on histopathology and are discussed below. Biological significance of gross tubule findings is not clear but male fertility and reproduction in rodents is not usually altered by modest physiological changes.

| Gross pathology summary † | | | | | |
|---|---|---|---|---|---|
| Organ/Tissue | Sex | Treatment (mg/kg/day) | | | |
| | | 0 | 8 | 25 | 80 |
| Lung (masses) | M | 20 | 30 | 29 | 26 |
| | F | 23 | 16 | 17 | 17 |
| Mandibular lymph nodes (enlarged) | M | 9 | 5 | 8 | 3 |
| | F | 7 | 10 | 8 | 14 |
| Adrenal (masses) | M | 5 | 8 | 9 | 12 |
| | F | 2 | 1 | 1 | 2 |
| Testes (prominent tubules) | M | 4 | 13 | 17 | 18 |

† Overall incidence in animals that died or were euthanized on study or at scheduled necropsy

### *Histopathology*

***Peer Review –*** Multiple levels of peer review were performed. The conducting laboratory provided a standard peer review by a separate pathologist. Lymphoma findings and brain slides (for benign and malignant meningiomas) were independently reviewed [redacted] (b) (4)

***Neoplastic –*** The most notable tumor finding was increased lymphoma (malignant) in female mice. Lymphoma incidences were 11, 4, 6, 0 (18%, 7%, 10%, 0%) in male and 6, 11, 11, and 22 (10%, 18%, 18%, 37%) in female respective dose groups. Statistical analyses confirmed a dose-response trend in the females ($p< 0.001$), with only female HD increased significantly compared to controls ($p< 0.001$, pairwise). The malignant nature of lymphoma was also evident with presence of lymphoma in many tissues across all groups and the trend of increased lymphomas in female HD consistent across the different tissues.

Lymphoma is commonly seen in mice, with female historical control incidence ranging from 17-28% in the conducting laboratory and higher in other laboratories (12-43% in RITA[14] database from multiple labs and 2-50% in [redacted] (b) (4) studies). The female HD percent incidence, 37%, was outside the conducting lab's historical range but within the upper range observed in other labs. In contrast, the concurrent control percent incidence, 10%, was below the conducting lab's historical range.

The Sponsor noted lymphoproliferative lesions in other hematopoietic/lymphoid tissues were not increased in either incidence or severity in treated females, including the high dose (see Sponsor's summary table, below). As shown in the tables, female lymphoid hyperplasia in spleen (8-27% treatment groups, not dose-related) and thymus (35-40% treatment groups, not dose-related) were similar or lower than the concurrent controls (23% in spleen, 48% in thymus). The Sponsor concluded that pre-neoplastic hyperplasia/proliferative lesions did not support a treatment related increase in lymphoma in female mice. However, it is also possible that tissue hyperplasia progressed to neoplasms in a higher percentage of HD females, thus resulting in an imbalance of non-neoplastic hyperplasia in HD compared to other groups.

The Sponsor further analyzed lymphoma onset and showed drug treatment did not decrease the time of onset to tumor formation (or at least tumor detection), providing further support that lymphoma were likely occurring spontaneously and correlated with age of mice more than drug exposure.

This reviewer agrees that lymphomas in mice are common and incidence is variable between studies, suggesting increased malignant lymphomas in HD females may be a spontaneous, incidental finding. However, the statistical analysis clearly showed a dose-response trend and significantly increased lymphomas in HD females compared to concurrent controls. Early deaths due to lymphoma and gross pathology observations of enlarged mandibular lymph nodes were also notably higher in HD females, consistent with the neoplastic trend and a drug-related increase in HD female lymphomas. The analysis provided by the Sponsor is not sufficient to dismiss the evidence of drug-related increased lymphomas in females under the conditions of the study. Drug-related lymphomas occurred only in HD females at approximately 215-times the MRHD. Estimating a NOAEL for drug-related neoplasms at 80 mg/kg/d for males and 25 mg/kg/d for females provide exposure margins of approximately 271X and 34X for males and females, respectively.

Statistical trends were identical in the FDA's independent statistical analyses (M.A. Rahman, 2/21/11). Also see the FDA statistics review for a list of combined tumors and a further discussion of tumor statistical analyses (M.A. Rahman, 2/21/11).

[14] *IBID*

**Summary of lymphoproliferative lesions in haemopoietic/lymphoid tissues**

| Group/sex<br>Dosage (mg/kg/day) | | 1M<br>0 | 2M<br>8 | 3M<br>25 | 4M<br>80 | 1F<br>0 | 2F<br>8 | 3F<br>25 | 4F<br>80 |
|---|---|---|---|---|---|---|---|---|---|
| Lymphoma | | | | | | | | | |
| | Incidence | 11 | 4 | 6 | 0 | 6 | 11 | 11 | 22 |
| | Percentage | 18.3 | 6.7 | 10.0 | 0.0 | 10.0 | 18.3 | 18.3 | 36.7 |
| Lymphoid hyperplasia - Spleen | | | | | | | | | |
| | Incidence | 9 | 10 | 3 | 6 | 17 | 16 | 15 | 5 |
| | Percentage | 15.0 | 16.7 | 5.0 | 10.0 | 28.3 | 26.7 | 25.0 | 8.3 |
| Number of spleens examined | | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| Lymphoid hyperplasia - Thymus | | | | | | | | | |
| | Incidence | 6 | 8 | 6 | 8 | 27 | 20 | 21 | 19 |
| | Percentage | 11.3 | 15.0 | 10.7 | 15.1 | 48.2 | 34.5 | 39.6 | 35.2 |
| Number of thymus examined | | 53 | 55 | 56 | 53 | 56 | 58 | 53 | 54 |

**Sponsor's time of lymphoma onset summary**

**Animals with malignant lymphomas: Mean age at necropsy in days**

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 8 mg/kg | 25 mg/kg | 80 mg/kg | Control | 8 mg/kg | 25 mg/kg | 80 mg/kg |
| Preterminal decedents | 539.5 | 396.3 | 444.7 | NA | 581 | 536.6 | 493.3 | 556.4 |
| All animals | 593.3 | 396.3 | 543.3 | NA | 581 | 554.3 | 581.5 | 556.4 |

NA, Not applicable because no malignant lymphomas were present.

In lung, both bronchioloalveolar adenoma and carcinoma were increased in male treatment groups but there was no dose-relationship and differences from controls were not statistically significant (either trend test or pairwise). Combined incidence of adenoma and carcinoma were also not statistically increased. The Sponsor noted HD tumor incidences were within the conducting lab's historical control range, but historical control data were not provided. The Sponsor's summary lung data are shown in the table below.

**Summary of selected neoplastic findings in the lung**

| Group/sex<br>Dosage (mg/kg/day) | | 1M<br>0 | 2M<br>8 | 3M<br>25 | 4M<br>80 | 1F<br>0 | 2F<br>8 | 3F<br>25 | 4F<br>80 |
|---|---|---|---|---|---|---|---|---|---|
| Bronchioloalveolar adenoma | | | | | | | | | |
| | Incidence | 13 | 16 | 20 | 17 | 12 | 7 | 7 | 10 |
| | Percentage | 21.7 | 26.7 | 33.3 | 28.3 | 20.0 | 11.7 | 11.7 | 16.7 |
| Bronchioloalveolar carcinoma | | | | | | | | | |
| | Incidence | 3 | 8 | 9 | 7 | 5 | 3 | 3 | 1 |
| | Percentage | 5.0 | 13.3 | 15.0 | 11.7 | 8.3 | 5.0 | 5.0 | 1.7 |
| Number of animals examined | | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |

Adrenal adenoma increased slightly in treated males, but incidences were not statistically different from controls for trend or pairwise comparisons. As noted above, gross adrenal hypertrophy, independent of dose, were also observed in males. Adrenal findings were not considered treatment related or biologically significant, particularly in the context of treatment not affecting overall survival.

**Summary of selected neoplastic findings in the adrenals**

| Group/sex<br>Dosage (mg/kg/day) | | 1M<br>0 | 2M<br>8 | 3M<br>25 | 4M<br>80 | 1F<br>0 | 2F<br>8 | 3F<br>25 | 4F<br>80 |
|---|---|---|---|---|---|---|---|---|---|
| Subcapsular cell adenoma | | | | | | | | | |
| | Incidence | 2 | 4 | 5 | 6 | 1 | 0 | 1 | 1 |
| | Percentage | 3.3 | 6.7 | 8.3 | 10.0 | 1.7 | 0.0 | 1.7 | 1.7 |
| Number of animals examined | | 60 | 60 | 60 | 60 | 59 | 60 | 60 | 60 |

Three brain tumors were seen in treatment groups but not in concurrent controls. Benign meningioma, fibrous type, was seen in 1/60 MD male and 1/60 HD female. Malignant meningioma, fibrous type, was seen in an additional 1/60 HD female. Thus, combining benign and malignant tumors there were 2/60 females with benign or malignant meningioma. The expected incidence was 0.43 meningioma (< 1%) based on historical control incidence. The Sponsor's statistical analyses showed p=0.045 for trend test, and p=0.177 for pairwise test. This brain tumor was not included in the Sponsor's list of 'rare' tumors, however, the Sponsor identified the tumor as rare in the discussion of neoplastic findings. Nevertheless, the incidence was not statistically significant for either a 'rare' or 'common' tumor, based on standard criteria (see statistical analysis comments, above). The Sponsor sent brain tumor slides to an independent pathologist for evaluation and additional peer-review. Neither the independent pathologist nor the Sponsor considered meningioma to be treatment related. This reviewer also considers the brain tumors to be incidental and unrelated to treatment, based on absence of a clear dose-response, late onset of each tumor (day 509 or later), and absence of statistical significance.

| Tissue and Finding | Group/Sex: Number: | 1M 60 | 2M 60 | 3M 60 | 4M 60 | 1F 60 | 2F 60 | 3F 60 | 4F 60 |
|---|---|---|---|---|---|---|---|---|---|
| Brain | Number Examined: | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| B-Meningioma, Fibrous Type | | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| M-Meningioma, Fibrous Type | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| N-Leukaemia | | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| N-Lymphoma | | 5 | 2 | 1 | 0 | 3 | 3 | 2 | 0 |

A 'rare', ovarian benign sertoliform tubular adenoma was seen in 2 HD females but no other females. The finding was not statistically increased for either trend or pairwise tests, whether the tumor was considered 'rare' or 'common'. The finding was considered incidental by the Sponsor and by this reviewer.

| Tissue and Finding | Group/Sex: Number: | 1M 60 | 2M 60 | 3M 60 | 4M 60 | 1F 60 | 2F 60 | 3F 60 | 4F 60 |
|---|---|---|---|---|---|---|---|---|---|
| Ovaries | Number Examined: | - | - | - | - | 60 | 59 | 60 | 60 |
| B-Cystadenoma | | - | - | - | - | 2 | 4 | 0 | 1 |
| B-Granulosa Cell Tumour | | - | - | - | - | 1 | 0 | 0 | 1 |
| B-Luteoma | | - | - | - | - | 2 | 1 | 3 | 4 |
| B-Mesovarian Leiomyoma | | - | - | - | - | 1 | 0 | 0 | 0 |
| B-Sertoliform Tubular Adenoma | | - | - | - | - | 0 | 0 | 0 | 2 |
| M-Choriocarcinoma | | - | - | - | - | 0 | 0 | 1 | 0 |
| N-Histiocytic Sarcoma | | - | - | - | - | 0 | 3 | 0 | 1 |
| N-Leukaemia | | - | - | - | - | 2 | 0 | 0 | 0 |
| N-Lymphoma | | - | - | - | - | 4 | 9 | 5 | 12 |
| N-Sarcoma | | - | - | - | - | 0 | 0 | 0 | 1 |

***Non-Neoplastic***

Incidence of fluid retention or accumulation seemed to be elevated in many tissues, based on findings such as edema, dilation, or dilatation. The findings were not likely treatment-related because findings were seen across all groups, but in several instances female HD incidence was slightly greater than control or other treatment groups (e.g., pancreas edema in 7, 6, 6, 13 females, respectively). Lymphoid aggregates were also noted in many tissues but there was no clear dose-response and background rates in controls were high.

Severity of testes germ cell depletion increased in treatment groups. The incidence of moderate germ cell depletion was higher than controls in all treatment groups, with the highest incidence in HD males. Overall incidence (any severity) was slightly higher in HD males. Slight testes mineralization was also seen in treated males, with no clear dose response. Total cases of minimal to slight mineralization were slightly higher in MD and HD males (10, 9, 14, 17, respectively). Additional male reproductive system findings included increased epididymids duct dilatation (1, 8, 6, 11, respectively), epithelial vacuolation (13, 18, 13, 19, respectively), and reduced numbers of spermatozoa (19, 25, 24, 31, respectively). The relationship of these histologic findings to gross observations of increased incidence of prominent tubules is not clear, but they may be related. The Sponsor did not consider male reproductive tissue findings to be toxicologically meaningful. This reviewer notes that the large number of findings in male reproductive tissues is noteworthy, however, it is not clear if findings were biologically significant. Male fertility was assessed separately in a dedicated rat study and there was no apparent drug-related effect. Testes findings are shown in the Sponsor's table, below.

**Summary of selected findings in the testes**

| Group/sex | | 1M | 2M | 3M | 4M |
|---|---|---|---|---|---|
| Dosage (mg/kg/day) | | 0 | 8 | 25 | 80 |
| Germ cell depletion | | | | | |
| | Minimal | 11 | 6 | 6 | 6 |
| | Slight | 13 | 7 | 8 | 7 |
| | Moderate | 4 | 11 | 8 | 22 |
| | Marked | 2 | 1 | 1 | 0 |
| | Total | 30 | 25 | 23 | 35 |
| Mineralisation | | | | | |
| | Minimal | 10 | 6 | 6 | 13 |
| | Slight | 0 | 3 | 8 | 4 |
| | Total | 10 | 9 | 14 | 17 |
| Number of animals examined | | 60 | 60 | 60 | 60 |

***Toxicokinetics –*** Drug was measured in treated groups with exposure increased greater than dose proportionally. Variability within groups was high, ranging from 13% to 88% for BI 1356 BS (coefficient of variation at $C_{max}$) and higher for metabolite CD 1750 XX. Exposure decreased slightly after repeated exposure, with approximately 25% lower exposure to drug on day 177. There was no consistent sex difference in exposure. CD 1750 XX exposure was approximately 1% of parent on day 177. Mean exposure data are shown in the Sponsor's summary tables, below.

Summary of mean toxicokinetic parameters of BI 1356 BS

| Parameter | Day | Gender | 8 mg/kg | 25 mg/kg | 80 mg/kg |
|---|---|---|---|---|---|
| $C_{(max)}$ [nmol/L] | 1 | m | 205 | 1590 | 9980 |
| | 1 | f | 344 | 1390 | 7070 |
| | 177 | m | 229 | 1520 | 9950 |
| | 177 | f | 184 | 1530 | 8400 |
| $AUC_{(0-24h)}$ [nmol·h/L] | 1 | m | 789 | 6520 | 59500 |
| | 1 | f | 1250 | 7220 | 47200 |
| | 177 | m | 765 | 4880 | 42600 |
| | 177 | f | 768 | 5470 | 33900 |

Summary of mean toxicokinetic parameters of CD 1750 XX

| Parameter | Day | Gender | 8 mg/kg | 25 mg/kg | 80 mg/kg |
|---|---|---|---|---|---|
| $C_{(max)}$ [nmol/L] | 1 | m | 2.13 | 21.0 | 111 |
| | 1 | f | 4.96 | 30.4 | 141 |
| | 177 | m | 2.86 | 19.5 | 85.2 |
| | 177 | f | 3.29 | 35.1 | 107 |
| $AUC_{(0\text{-}24h)}$ [nmol·h/L] | 1 | m | 11.1 | 125 | 837 |
| | 1 | f | 20.2 | 173 | 1440 |
| | 177 | m | 6.28 | 70.8 | 406 |
| | 177 | f | 15.8 | 168 | 678 |

***Stability and Homogeneity –*** Stock assay solutions were tested appropriately for stability, homogeneity, and drug concentration. Mean concentrations ranged from 87.3% to 99.2% of nominal concentrations. Various individual samples were outside the ± 10% nominal range but deviations were infrequent and did not affect the integrity of the study over the 2-year dosing period. Neither drug nor major metabolite were detected in control group samples.

# 9 Reproductive and Developmental Toxicology

## 9.1 Fertility and Early Embryonic Development

**Fertility and early embryonic development in at ('Segment 1')**

**BI 1356 BS: Study of fertility and early embryonic development to implantation in rats by oral administration, gavage (05B189; Doc. No. U06-2047)**

*Doses* *0, 10, 30, 240 mg/kg/d*

***NOAEL (Male)*** *= 240 mg/kg (~800X MRHD estimated from chronic rat study)*
***NOAEL (Female)*** *= 240 mg/kg (158,000 nM*h estimated from Seg. 2; 1000X MRHD)*

***NOAEL determination –*** Treatment with 240 mg/kg prior to mating did not have any apparent effect on fertility based on the absence of any reproductive or embryofetal toxicity.

**Key study findings**:

- The NOAEL for reproductive and early embryo-fetal toxicity in rats was > 240 mg/kg/day BI 1356 BS based on the absence reproductive or embryofetal toxicity at the highest dose of 240 mg/kg/day.

- At 240 mg/kg/day, there was evidence of slight toxicity in sexually mature male and female rats during the pre-mating treatment phase characterized by significantly reduced body weight gain and decreased food consumption compared to concurrent controls. However, reduced body weight gain was not reflected in statistically significantly lower body weight, and in females, it was not observed throughout the entire pre-mating period.

- In the absence of reporting histopathology of testes or effects on spermatogenesis in treated males, conclusions regarding the lack of an effect of BI 1356 BS on spermatogenesis based on the fertility index, which was unaffected by treatment, are unsubstantiated. The ability of a male rat to impregnate a female is an insensitive indicator of effects on spermatogenesis.

- No TK analyses were performed but post-dose clinical signs and BW gain decreases in HD animals confirmed drug exposure. Histopathology of reproductive tissues of treated animals whose partner was not pregnant at necropsy were unremarkable, but there was no dedicated analysis of spermatogenesis.

## 9.2 Embryonic Fetal Development

Embryofetal development was investigated in non-GLP dose-ranging studies in rat and rabbit, followed by definitive GLP compliant studies. All study reports were fully reviewed but results from range-finding assays are only discussed in the context of findings from definitive studies.

### Embryofetal development in rat ('Segment 2')

**BI 1356 BS: Study for effects on embryo-fetal development in rats by oral (gavage) administration (04B226 A1; Doc. No. U06-1637)**

*Doses:* *0, 10, 30, 240 mg/kg/d*
*Exposure:* *2090; 7,710; 158,000 nM*h (MD ♀ exposure estimated from GD 7)*

***NOAEL (maternal)*** *= 30 mg/kg (49X MRHD)*
***NOAEL (fetal)*** = *30 mg/kg (49X MRHD)*

***NOAEL determination –*** The NOAEL for maternal and embryofetal toxicity was 30 mg/kg/day linagliptin based on HD reduced maternal BW gain and reduced BW compared to concurrent controls concomitant with decreased food consumption. HD linagliptin also resulted in slightly (nss) decreased number of corpora lutea, embryofetal survival and increased late resorptions. Various skeletal variations, generally associated with delayed ossification, were increased across treatment groups but were not clearly associated with or predictive of skeletal malformations. There were slight increases in skeletal malformations above concurrent controls but the *final NOAEL considered LD and MD findings to be unrelated to treatment based on absence of a clear dose-response, historical control findings, and absence of any malformations in MD above the historical range. Rib malformations (flat and thickened) in HD were only slightly outside historical controls, but they were potentially drug-related because a 2.5-fold higher dose caused a very high incidence of the same rib malformations in the range-finding assay.*

**Key study findings**:

- The NOAEL for embryofetal toxicity was 30 mg/kg/day. Skeletal variations and malformations were increased slightly above concurrent controls at 10 mg/kg, but not 30 mg/kg, and findings were not considered adverse based on similar incidence in controls in the range-finding assay and similar background incidence in historical controls.

- Increased embryofetal intrauterine mortality occurred at 240 mg/kg/day BI 1356 BS characterized by changes in litter parameters of decreased number of implantations and viable fetuses and increased late resorptions and pre-implantation loss.

- Treatment was associated with various skeletal variations consistent with delayed ossification and delayed development but not clearly associated with malformations expected to affect embryofetal survival.

- Three skeletal malformations were slightly increased compared to concurrent controls – cleft cervical vertebral body, cleft thoracal vertebral body, and flat and thickened ribs. There was no clear relationship to dose, no clear increase in liter incidence, and no clear increased incidence outside the historical range. Incidence of flat and thickened ribs was 57% in the range-finding assay at a very high dose (600 mg/kg) compared to 13.5% in the concurrent controls, suggesting the 17% incidence at 240 mg/kg in the definitive study may indicate the lower end of a treatment-related increase in rib malformations.

- A dosing error occurred on GD16 and prevented accurate TK exposure collection. Exposure after the initial dose on GD 7 was used as an estimate of total exposure in the MD dams on GD16.

- Linagliptin was detected in control group dams on GD 7, which had the potential to cause time-dependent toxicity in the developing animals. Overall, control findings relevant to potential drug-induced embryofetal toxicity were considered by this reviewer to be generally consistent with historical control findings.

## Embryofetal development in rabbit (‘Segment 2’)

**BI 1356 BS: Study for effects on embryo-fetal development in rabbits by oral (gavage) administration (05B097; Doc. No. U06-1200)**

*Doses:* *0, 4, 25, 150 mg/kg/d*
*Exposure:* *339; 12,400; 307,000 nM*h*

***NOAEL (maternal)*** *= 25 mg/kg (78X MRHD)*
***NOAEL (fetal)*** *=* *150 mg/kg (fetal malformations; 1943X MRHD)*
*< 4 mg/kg (fetal variations; 2X MRHD)*

***NOAEL determination –*** Maternal and embryofetal toxicity were evident in the HD based on increased resorptions and decreased BW gain concomitant with decreased food consumption. There were no treatment-related fetal malformations. Fetal variations of small gall bladder/hypoplasia and increased lumbar ribs (summa) were increased in LD and HD compared to concurrent and historical controls. The absence of a dose-response due to absence of similar variations in the MD, coupled with the relatively common occurrence of gall bladder abnormalities in fetal rabbits, suggests LD findings may not be biologically significant.

**Key study findings**:

- There were no malformations considered treatment-related.

- The HD of 150 mg/kg/d increased the number of early and total resorptions and the resorption rate.

- The NOAEL for maternal toxicity was 25 mg/kg/day BI 1356 BS based on 39% decreased body weight gain compared to controls on gestation day 28 and reduced food consumption during the period of drug administration, study weeks 2 and 3, at 150 mg/kg/day. Although decreased body weight gain may be due to decreased litter size in the 150 mg/kg/day group, at least in part, decreased food consumption during the period of drug administration supports maternal toxicity.

- The NOAEL for embryo-fetal toxicity was < 4 mg/kg/day BI 1356 BS based on increased incidence of fetal variations of small gall bladder/gall bladder hypoplasia. and increased lumbar ribs (summa) in LD and HD. The MD variations were not increased over concurrent controls or the historical control range.

## Linagliptin + metformin combination rat embryofetal development ('Segment 2')

**BI 1356 BS (Linagliptin) and metformin: Study for effects on embryo-fetal development in rats by oral (gavage) administration (09B138 A1; Doc. No. U10-2448-01)**

| | |
|---|---|
| *Doses* | *0/0 (vehicle control)* |
| *(mg/kg/d)* | *5/0 (linagliptin/metformin)* |
| | *0/1000* |
| | *1/200, 2.5/500, 5/1000, 2.5/1000* |

***NOAEL (maternal)*** *= < 1/200 (5/0 linagliptin, < 0/1000 metformin controls)*
***NOAEL (fetal)*** = *< 1/200 (5/0 linagliptin, < 0/1000 metformin controls)*

***Summary and NOAEL determination –*** A combination linagliptin plus metformin combination embryofetal rat study was conducted to determine any potential additive or synergistic toxicity between the antidiabetic agents. A cursory review confirmed the absence of teratogenic effects in the linagliptin control group (5 mg/kg). The linagliptin dose was slightly lower than the pivotal rat embryofetal development LD group, but results support the conclusion that there were no LD drug-related teratogenic findings in the linagliptin rat reproductive toxicity studies.

## 9.3 Prenatal and Postnatal Development

**Rat pre- and postnatal development (Segment 3)**

**Study for effects on pre- and postnatal development including maternal function in rats by oral administration, gavage (Study 05B241; Doc. No. 07-1558)**

*0, 10, 30, 300 mg/kg (0.5% hydroxyethylcellulose)*
*1.2, 7.7, 186 μM*h (estimated from GD 7 dosing in pregnant rats, Doc. U06-1637)*

***NOAEL (maternal)*** *= 30 mg/kg (49X MRHD)*
***NOAEL (fetal)*** = *30 mg/kg (24X MRHD estimated based on 50% fetal exposure compared to maternal exposure)*

***NOAEL determination –*** Findings in $F_0$ and $F_1$ rats treated during pregnancy ($F_0$) and *in utero* and throughout lactation ($F_1$) suggest the HD treatment of 300 mg/kg (> 1000X MRHD) linagliptin resulted in delayed growth and development of offspring. HD-treated pregnant rats had treatment-related decreased body weight gain and decreased body weight compared to controls. Delayed labor and postimplantation loss were increased in HD dams. Body weights of offspring ($F_1$) from HD dams were decreased at birth and remained lower than controls throughout adolescence, mating, and pregnancy. HD offspring ($F_1$) also had modest delays in physical development, learning/memory, physical activity/exploratory behavior, and in number of viable offspring ($F_2$) after mating. Mating, fertility, and pregnancy of $F_1$ animals were generally otherwise unaffected by the *in utero* and lactational exposure to the HD of drug. Fetal and neonatal exposure were not measured in this study but separate studies confirmed linagliptin crosses the placenta to expose developing fetuses and it is excreted in milk at 4-times the concentration of maternal plasma.

**Key study findings**:

- HD $F_0$ dams had decreased BW and BW gain during pregnancy (concomitant with decreased food consumption) during pregnancy) and BW remained lower throughout lactation until LD 20
- Delayed labor in 5/21 HD $F_0$ dams and slightly increased number of dead offspring (2 dead in 2 different litters)
- Increased $F_0$ postimplantation loss (16% HD vs. 7% control)
- No treatment effects on overall $F_1$ viability or weaning rate
- BW decreased slightly (7%) in $F_1$ pups, persisted throughout development and remained decreased at $F_1$ mating

- o Rate of weight gain in $F_1$ after birth was similar to other groups, but lower birth weights contributed to BW differences that persisted throughout adolescence into adulthood

- General delay in physical development in HD $F_1$ (fur growth, eye opening, male preputial separation), consistent with slightly lower BW and a general delayed growth

- HD $F_1$ had slight delays in learning and slight decreases in physical activity/exploratory behavior

- $F_1$ mating, fertility, and pregnancy were generally successful

- Slight decrease in $F_1$ reproductive success as measured by the number of viable $F_2$ fetuses, consistent with slightly lower $F_1$ maternal BW

- Pre- and postnatal findings in the study were consistent with decreased BW and general developmental delays which occurred only at very high multiples of expected human exposure. There was no evidence of direct drug-related toxicity (i.e., other than BW or growth delays) on developing embryos or that markedly affected reproductive success of offspring exposed *in utero* and throughout lactation.

| | |
|---|---|
| Study no: | 05B241; Doc. No. 07-1558 |
| Study report location: | eCTD 4.2.3.5.3 |
| Conducting laboratory and location: | Boehringer Ingelheim Pharma GmbH & Co. KG<br>Birkendofer Str. 65<br>88397 Biberach an der Riss<br>Germany |
| Date of study initiation: | 1/5/06 (animal acclimatization) |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | BI356BS, Batch #3050850, 97.4% purity |

**Methods**

| | |
|---|---|
| Doses: | 0, 10, 30, 300 mg/kg |
| Frequency of dosing: | Daily gestation days 6 (GD6) to lactation day 21 (LD21) |
| Dose volume: | 10 ml/kg |
| Route of administration: | Oral gavage |
| Formulation/Vehicle: | 0.5% hydroxyethylcellulose (Natrosol® 250 HX) |
| Species/Strain: | Wistar Han rat |
| Number/Sex/Group: | 24 pregnant females/group |
| Satellite groups: | None |
| Study design: | Mated dams ($F_0$) treated GD 6 to LD 21 (through remaining pregnancy and nursing until weaning of $F_1$ pups); **$F_0$ parameters –** gestation duration, litter size, stillbirths, live births, gross abnormalities; clinical signs, BW, food consumption; **$F_1$ parameters –** clinical signs, BW, food consumption; maturation signs (incisor eruption LD 11, fur growth LD 13, ear canal LD 13 & eye opening LD 15, correct running LD 13, descensus testis LD 21, vaginal opening PND 36, preputial separation PND 44), physiological function Week 4 (pupillary reflex, righting reflex, hearing), behavior Week 6 & 7 (Biel water T-maze test), spontaneous activity Week 5 (open field, photobeam activity system); **$F_1$ reproduction –** offspring were mated 1:1 within treatment groups (sibling matings avoided) at 10-12 weeks old, BW (days 1, 7, 14 post-coitus (p.c.), reproduction and fertility assessed (copulation index, fertility index, gestation index), and pregnancy parameters and early reproduction through GD 14 (corpora lutea, implantations, live/dead fetuses, resorptions); males were killed and necropsied after mating (reproductive organs – testes, epididymids, prostate, seminal vesicles – examined histologically in males with failed matings); pituitary glands were examined histologically in mated, non-pregnant females **$F_2$ observations –** fetal examinations were limited to assessment of survival (live vs. dead) on GD 14 of $F_1$ females; no assessment of $F_2$ fetal development |
| Deviation from study protocol: | Minor deviations not expected to affect study validity or integrity |

**Sponsor's Study Design Summary**

| Group | Females per group | Weighed test article | Dose [mg/kg] | Conc. [%] | Animal Nos. |
|---|---|---|---|---|---|
| 1 | 24 | Natrosol 250 HX (0.5%) | 0 | 0 | 101-124 |
| 2 | 24 | BI 1356 BS | 10 | 0.10 | 201-224 |
| 3 | 24 | BI 1356 BS | 30 | 0.30 | 301-324 |
| 4 | 24 | BI 1356 BS | 300 | 3.00 | 401-424 |

**Observations and Results:**

**$F_0$ Dams** (additional observations not noted in study design above)

Toxicokinetics: 1.2, 7.7, and 186 µM*h BI 1356 BS

Dosing Formulation Analysis: Analyses conducted and found to be acceptable

**$F_1$ Generation** (additional observations not noted in study design above)

Survival: Viability index determined on LD 4 (four days after delivery); weaning index determined LD 21

Other: Litters culled on LD 4 to provide 5 pups/sex/litter through lactation; culled to 2 pups/sex/litter on LD 21

**$F_2$ Generation** (additional observations not noted in study design above)

Other: Observations were limited to assessment of survival on $F_1$ GD 14. No assessment of gross external development or sex ratio were conducted.

<u>**$F_0$ Dams**</u>

**Mortality –** No treatment-related effects on survival during pregnancy. One LD dam was killed on GD 10 after BW loss through early pregnancy (prior to treatment), with cause of morbidity attributed to pyelonephritis and urinary crystals in the right kidney.

**Clinical Signs** – Salivation in 9/24 HD dams after multiple days of dosing (beginning GD 15 and continuing through lactation).

**Body Weight** – HD dams had decreased BW and decreased BW gain compared to controls throughout treatment during pregnancy. BW remained decreased through most of lactation until LD 20. Maternal BW gain was increased in the HD group during lactation to the extent that by LD 20, HD maternal BW was no longer statistically lower than controls. BW gain in LD and MD groups was increased at the start of the lactation period (through LD 5) but did not result in increased mean BW compared to control BW. Data are summarized in the Sponsor's summary tables, below.

### Sponsor's body weight and body weight gain summary tables

BI 1356 BS: Study for effects on pre- and postnatal development including maternal function in rats - mean of body weight [g]

| Dose [mg/kg] | n dams | Mean of body weight [g] during gestation GD 1 | GD 7 | GD 8 | GD 9 | GD 16 | GD 19 | GD 22 |
|---|---|---|---|---|---|---|---|---|
| Control | 20 | 203.99 | 223.54 | 226.74 | 229.86 | 254.59 | 279.00 | 305.44 |
| 10 | 18 | 202.61 | 220.69 | 223.88 | 226.49 | 251.10 | 275.89 | 307.29 |
| 30 | 19 | 203.27 | 221.99 | 224.76 | 227.24 | 254.05 | 280.56 | 314.01 |
| 300 | 21 | 202.92 | 219.32 | 222.32 | 223.10*↓ | 239.84*↓ | 260.63*↓ | 288.20*↓ |

| Dose [mg/kg] | n dams | Mean of body weight [g] during lactation LD 1 | LD 2 | LD 4 | LD 5 | LD 9 | LD 19 | LD 20 | LD 21 |
|---|---|---|---|---|---|---|---|---|---|
| Control | 19### | 239.85 | 236.65 | 247.56 | 252.44 | 267.21 | 279.59 | 277.05 | 277.82 |
| 10 | 18## | 230.99*↓ | 233.47 | 244.41 | 249.32 | 265.98 | 274.71 | 275.29 | 272.39 |
| 30 | 19 | 232.38 | 235.84 | 248.80 | 252.51 | 266.24 | 277.07 | 277.66 | 274.39 |
| 300 | 20# | 223.46*↓ | 224.43*↓ | 232.06*↓ | 238.64*↓ | 251.18*↓ | 270.95*↓ | 271.10 | 270.47 |

# dam No. 423 excluded (implantation sites only, all offspring swallowed)
## body weight of dam No. 214 was not registered on LD 1
### dam No. 107 was only registered on LD 1 and 2 (all offspring swallowed)
* significant difference ($p<0.05$)
↓ decreased
GD = gestation day
LD = lactation day (equals postnatal day [PND])

BI 1356 BS: Study for effects on pre- and postnatal development including maternal function in rats - mean of body weight gain [g]

| Dose [mg/kg] | n dams | Mean of body weight gain [g] during gestation relative to GD 6 GD 7 | GD 8 | GD 10 | GD 16 | GD 19 | GD 22 |
|---|---|---|---|---|---|---|---|
| Control | 20 | 2.87 | 6.07 | 11.70 | 33.92 | 58.33 | 84.77 |
| 10 | 18 | 1.63 | 4.82 | 10.90 | 32.04 | 56.83 | 88.23 |
| 30 | 19 | 1.46 | 4.23*↓ | 10.73 | 33.52 | 60.03 | 93.47 |
| 300 | 21 | -1.57*↓ | 1.43*↓ | 4.40*↓ | 18.96*↓ | 39.75*↓ | 67.32*↓ |

| Dose [mg/kg] | n dams | Mean of body weight gain [g] during lactation relative to LD 1 LD 2 | LD 4 | LD 5 | LD 9 | LD 18 | LD 19 | LD 20 | LD 21 |
|---|---|---|---|---|---|---|---|---|---|
| Control | 19### | -3.20 | 7.28 | 12.16 | 26.93 | 39.39 | 39.31 | 36.77 | 37.54 |
| 10 | 17## | 2.52*↑ | 13.66*↑ | 18.45 | 35.22 | 45.16 | 44.52 | 44.64 | 41.55 |
| 30 | 19 | 3.46*↑ | 16.42*↑ | 20.13*↑ | 33.86 | 47.56*↑ | 44.69 | 45.28*↑ | 42.01 |
| 300 | 20# | 1.29 | 8.91 | 15.49 | 28.04 | 46.16 | 47.81*↑ | 47.96*↑ | 47.32*↑ |

# dam No. 423 excluded (implantation sites only, all offspring swallowed)
## body weight of dam No. 214 was no registered on LD 1
### dam No. 107 was only registered on LD 1 and 2 (all offspring swallowed)
* significant difference ($p<0.05$)
↓ decreased, ↑ increased
GD = gestation day
LD = lactation day (equals postnatal day [PND])

**Feed Consumption** – Food consumption decreased in HD dams during the treatment period during pregnancy. Food consumption remained slightly lower in HD females during lactation, but as noted above mean BW gain increased during lactation in the HD group.

### Sponsor's maternal food consumption summary

BI 1356 BS: Study for effects on pre- and postnatal development including maternal function in rats - mean of food consumption [g]

| Dose [mg/kg] | n dams | Food consumption, mean [g] during pregnancy | | |
|---|---|---|---|---|
| | | GD 6 | GD 14 | GD 21 |
| Control | 20 | 86.43 | 155.72 | 145.88 |
| 10 | 18 | 86.31 | 151.01 n=17 | 145.45 |
| 30 | 19 | 85.83 | 149.63 | 144.92 |
| 300 | 21 | 87.91 | 121.77*↓ n=20 | 125.50*↓ |

| Dose [mg/kg] | n dams | Food consumption, mean [g] during lactation | | | |
|---|---|---|---|---|---|
| | | LD 4 | LD 7 | LD 14 | LD 21 |
| Control | 19### | 76.34 | 118.21 | 367.49 | 444.21 |
| 10 | 18 | 83.84## | 116.84 | 370.89 | 441.80 |
| 30 | 19 | 80.73 | 121.31 | 372.15 | 450.18 |
| 300 | 20# | 79.80 | 112.14 | 338.24*↓ | 423.26 |

# dam No. 423 excluded (implantation sites only, all offspring swallowed)
## food consumption of dam No. 214 was not registered on LD 4, n=17 on LD 4
### dam No. 107 excluded (all offspring swallowed)
* significant difference ($p<0.05$)
↓ decreased
GD = gestation day
LD = lactation day (equals postnatal day [PND])

**Toxicokinetics –** Toxicokinetics were not measured in the current study. Exposure was estimated from pregnant dams in a prior embryofetal development study (Study 04B226, Doc. No. 06-1637). Estimated exposures used for this review were 1.2, 7.7, and 186 µM*h BI 1356 BS estimated from initial dosing on GD 7 (see Sponsor's TK summary table, below).

### Sponsor's TK summary

BI 1356 BS: Study for effects on embryo-fetal development in rats (Viertel et a. 2006, U06-1637) - calculated mean C(max) and mean AUC(0-24h) values

| Parameter | Gestation day* | 0 mg/kg | 10 mg/kg | 30 mg/kg | 240 mg/kg |
|---|---|---|---|---|---|
| C(max) [nmol/L] | 7 | 5.97 | 171 | 1230 | 10900 |
| | 16 | 0 | 553 | 350# | 11900 |
| AUC(0-24h) [nmol·h/L] | 7 | - | 1190 | 7710 | 149000 |
| | 16 | - | 2090 | 2120# | 158000 |

*: GD 7 = Day 1 of dosing; GD 16 = Day 10 of dosing
#: dose erroneously administered in two fractions within 0.5 h

### Necropsy (Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.) –

Pregnancy success was not affected by treatment. At necropsy 20, 18, 19, and 21 dams were pregnant in respective groups. Various dams in different groups, independent of treatment, did not effectively rear young. Gross and histological lesions were seen at necropsy in some of the females, none of which were considered treatment related.

Dams that delivered on GD 23 rather than expected GD 22 (i.e., delayed labor) increased in the HD group (5/21 dams) compared to control and other groups (2/20, 1/18, 1/19, respectively). Mean post-implantation loss increased in the HD (16% vs. 6.6% controls). 2/21 HD pregnant dams had dead offspring (1 per dam) and 1/21 HD dams was found to have swallowed 3 offspring after birth (viability at birth not determined). No other dead offspring were detected at birth, but 1/21 control dams gave birth to a single offspring that was swallowed on LD 2. The control dam had no visible nipples macroscopically and nipples were found to be inverted at necropsy.

## Sponsor's pregnancy summary ($F_1$)

BI 1356 BS: Study for effects on pre- and postnatal development including maternal function in rats - litter and offspring parameters

| | G1 | G2 | G3 | G4 | Control group from a study for effects on pre- and postnatal development [U06-1586, Viertel et al. 2006] | Spontaneous incidences from an evaluation study for effects on embryo-fetal development [U03-1549, Viertel, 2003] | |
|---|---|---|---|---|---|---|---|
| | Control | 10 mg/kg | 30 mg/kg | 300 mg/kg | | | |
| n litters + | 20 | 18 | 19 | 21## | 22 | 85 | |
| Offspring characteristics at delivery (means/individual range per dam) | | | | | | means | means/ individual data |
| Implantations | 10.4/6-14 | 11.3/6-14 | 11.6/5-13 | 11.3/3-14 | 11.4/1-15 | 11.1 | 10.9-11.4/ 5.0-15.0 |
| Newborn offspring | 9.6/1-12 | 10.6/6-14 | 11.3/4-13 | 9.9/3-13 | 10.8/1-15 | 10.5 | 10.1-10.7/ 5.0-14.0 |
| Dead offspring | 1§/1-0 | 0 | 0 | 5§/0-3<br>2§/0-1### | 0.090/0-1 | 0 | 0 |
| Birth index [%] | 93.44/ 72.73-100 | 93.85/ 66.67-100 | 96.82/ 80.00-100 | 88.05/ 60.00-100### | 95.18/81.82-100 | - | - |
| Sex of viable offspring at birth (%) | | | | | | | |
| Male | 46.10/ 0-90.00 | 50.03/ 30.77-80.00 | 51.33/ 18.18-100 | 50.31/ 23.08-70.00### | 50.85/22.22-100 | 49.41 | 46.92-52.18/ 18.18-80.0 |
| Female | 53.90/ 100-10.00 | 49.97/ 20.00-69.23 | 48.67/ 0-81.82 | 49.69/ 30.00-76.92### | 49.15/0-77.78 | 50.59 | 47.82-53.08/ 20.0-81.82 |
| Post-implantation loss [%] | 6.56/ 0-27.27 | 6.15/ 0-33.33 | 3.18/ 0-20.00 | 16.14*↑/ 0-100 | 4.82/0-18.18 | resorption rate (%)<br>5.70 | <br>2.30-7.29/ 0[7.69]-23.08 # (54.55) |
| Length of gestation period, n dams (percentage of dams, decimals truncated) | | | | | | | |
| 22 days | 18 (90) | 17 (94.4) | 18 (94.7) | 16 (76.1)<br>16 (80)### | 20 (90.90) | - | - |
| 23 days | 2 (10) | 1 (5.5) | 1 (5.2) | 5 (23.8)<br>4 (20)### | 2 (9.09) | - | - |

* significantly different to control ($p<0.05$)
↑ increased
+ non-pregnant animals excluded
# one outlayer (No. 319) excluded (in brackets: No. 319 included); mean calculated with the outlayer included
[ ] the lowest number greater than 0
## dam No. 423 included (implantation sites only, all three offspring swallowed), influencing the range of dead offspring (see `Discussion)
### dam No. 423 excluded
§ absolute value

## $F_1$ Generation

**Survival/Mortality –** 2/21 HD pregnant dams had dead offspring (1 per dam) and 1/21 HD dams was found to have swallowed 3 offspring after birth (viability at birth not determined). A single runt (weighing < 65% of mean pup weight) was birthed from a HD female. 1/21 control dams gave birth to a single offspring that could not feed due to absence/inverted nipples in the dam. A few pups died perinatally after live birth (1, 0, 1, 2, respectively), some of which had macroscopic variations or malformations. Several additional pups died or were swallowed postnatally (2, 0, 6, 1). There was no dose-relationship or apparent overall treatment effect on viability (99.48%, 99.47%, 97.66%, 99.52%, respectively) or weaning rate (99.39%, 100%, 98.8%, 99.44%, respectively).

There were no apparent treatment-related effects on embryofetal development in the $F_1$ generation, based on necropsy of dead animals or pups culled on scheduled days (LD 4 and LD 21).

With the exception of the possible drug-related effect on the slight increase in number of dead (or swallowed) pups at birth in the HD, no other effects were considered drug related.

**Clinical Signs** – No treatment-related clinical signs.

**Body Weight** – $F_1$ HD body weights were reduced approximately 7% (ss) at birth and remained significantly lower than controls throughout lactation (↓ 12% on LD 21) and weaning (↓6-7% in females and males on postnatal day 70). In mated (presumed pregnant) $F_1$ females, maternal BW also remained 6-7% lower than controls throughout pregnancy. $F_1$ body weight gain trends were generally similar to BW trends. BW gain trends in $F_1$ females showed that absolute weight gain after weaning (PND 21) and throughout pregnancy were similar to controls, even though overall weight gain from birth remained consistently lower than controls (see Sponsor's BW gain table 'relative to PND 21' and 'relative to GD1'). Thus, data show decreased BW in offspring of $F_0$ females treated during pregnancy and lactation was essentially fixed in the $F_1$ generation, but $F_1$ animals generally gained weight at a similar rate as other groups.

## Sponsor's $F_1$ body weight and weight gain summaries

| Dose [mg/kg] | n litters | Mean of body weight [g] during lactation | | | | |
|---|---|---|---|---|---|---|
| | | LD 1[+] | LD 4 | LD 7 | LD 14 | LD 21 |
| Control | 19 | 5.743## | 8.552 | 13.703 | 28.481 | 43.139 |
| 10 | 18 | 5.605### | 8.602 | 13.785 | 28.136 | 42.710 |
| 30 | 19 | 5.781 | 8.653 | 14.137 | 28.938 | 44.074 |
| 300 | 20[#] | 5.358*↓ | 7.840*↓ | 12.222*↓ | 24.633*↓ | 37.940*↓ |

| Dose [mg/kg] | n litters | Mean of body weight gain [g] during lactation relative to LD 1 | | | |
|---|---|---|---|---|---|
| | | LD 4 | LD 7 | LD 14 | LD 21 |
| Control | 19 | 2.876 | 8.027 | 22.804 | 37.462 |
| 10 | 17### | 2.781 | 7.883 | 21.971 | 36.284 |
| 30 | 19 | 2.872 | 8.356 | 23.157 | 38.292 |
| 300 | 20[#] | 2.482*↓ | 6.864*↓ | 19.275*↓ | 32.582*↓ |

[+] day of birth
[#] dam No. 423 excluded (implantation sites only, offspring swallowed)
* significant difference ($p<0.05$)
↓ decreased
LD = lactation day (equals postnatal day [PND])
## on LD 1 dam No. 107 included (all offspring swallowed on LD 2)
### on LD 1 dam No. 214 excluded

| Dose [mg/kg] | Mean of body weight [g] of male offspring after weaning | | | | | | |
|---|---|---|---|---|---|---|---|
| | n litters | PND 28 | n litters | PND 35 | PND 56 | PND 63 | PND 70 |
| Control | 18 | 75.147 | 19 | 119.945 | 246.555 | 278.324 | 302.663 |
| 10 | 15 | 74.690 | 18 | 121.178 | 248.931 | 280.844 | 307.119 |
| 30 | 19 | 76.700 | 19 | 120.574 | 241.624 | 270.982 | 289.100 |
| 300 | 18 | 67.381*↓ | 20 | 109.168*↓ | 227.905*↓ | 257.968*↓ | 282.165*↓ |

| Dose [mg/kg] | Mean of body weight [g] of female offspring after weaning | | | | | Mean of body weight [g] of female offspring during pregnancy | | | |
|---|---|---|---|---|---|---|---|---|---|
| | n litters | PND 28 | n litters | PND 56 | PND 70 | n litters | GD 1 | GD 7 | GD 14 |
| Control | 18 | 69.475 | 19 | 171.271 | 193.926 | 16 | 211.01 | 231.12 | 253.29 |
| 10 | 15 | 68.510 | 18 | 169.333 | 193.133 | 16 | 208.81 | 227.49 | 251.11 |
| 30 | 18 | 71.336 | 18 | 169.678 | 192.603 | 16 | 210.11 | 228.59 | 252.01 |
| 300 | 18 | 63.606*↓ | 20 | 162.790*↓ | 183.035*↓ | 19 | 195.72*↓ | 215.35*↓ | 237.77*↓ |

* significant difference ($p<0.05$)
↓ decreased
PND = postnatal day, GD=gestation day

| Dose [mg/kg] | Mean of body weight gain [g] of male offspring after weaning relative to PND 21 | | | | | | |
|---|---|---|---|---|---|---|---|
| | n litters | PND 28 | n litters | PND 35 | PND 56 | PND 63 | PND 70 |
| Control | 18 | 31.528 | 19 | 76.021 | 202.632 | 234.400 | 258.740 |
| 10 | 15 | 31.708 | 18 | 77.540 | 205.293 | 237.207 | 263.482 |
| 30 | 19 | 31.882 | 19 | 75.756 | 196.806 | 226.164 | 244.282*↓ |
| 300 | 18 | 29.255*↓ | 20 | 70.759*↓ | 189.497*↓ | 219.559*↓ | 243.757*↓ |

| Dose [mg/kg] | Mean of body weight gain [g] of female offspring after weaning relative to PND 21 | | | | | Mean of body weight gain [g] of female offspring during pregnancy relative to GD 1 | | |
|---|---|---|---|---|---|---|---|---|
| | n litters | PND 28 | n litters | PND 56 | PND 70 | n litters | GD 7 | GD 14 |
| Control | 18 | 27.366 | 19 | 128.894 | 151.549 | 16 | 20.11 | 42.28 |
| 10 | 15 | 27.243 | 18 | 127.543 | 151.343 | 16 | 18.68 | 42.30 |
| 30 | 18 | 28.668 | 18 | 127.010 | 149.935 | 16 | 18.49 | 41.91 |
| 300 | 18 | 26.438 | 20 | 125.350 | 145.595 | 19 | 19.64 | 42.05 |

* significant difference ($p<0.05$)
↓ decreased
PND = postnatal day, GD=gestation day

**Physical Development –** Physical development parameters showed slightly delayed development in $F_1$ HD animals for fur growth, eye opening, and preputial separation (males). Testis descensus was delayed in all male groups including controls compared to historical controls (descensus expected PND 21). HD male testis descensus seemed slightly delayed compared to controls but the difference was not statistically significant and percent descensus by PND 24 was greater than controls. Trends for both delayed preputial separation and testis descensus suggested the delays were correlated with lower BW. All of the HD physical development delays were consistent with a general delay in growth (and consistent with decreased birth weight and persistently lower BW and BW gain) rather than a direct-acting effect of drug on developmental endpoints. Nevertheless, physical development delays were consistent with a general drug-induced effect on $F_1$ growth and development due to HD exposure during fetal development and nursing. The Sponsor's summary data are shown below.

## Sponsor's physical development summary

BI 1356 BS: Study for effects on pre- and postnatal development including maternal function in rats – postnatal physical parameters of the offspring, frequency distribution of time point of occurrence

| | Control | | 10 mg/kg | | 30 mg/kg | | 300 mg/kg | | Control group from a study for effects on pre- and postnatal development [U06-1586, Viertel et al. 2006] | |
|---|---|---|---|---|---|---|---|---|---|---|
| n litters | 19 | | 18 | | 19 | | 20 | | 22 | |
| Day of onset | Frequency [%] of offspring showing a physical landmark on scheduled day of onset | | | | | | | | | |
| Incisors eruption (scheduled day 11) | | | | | | | | | | |
| < 12 | 62.8 | Σ 94.5 | 81.1*↑ | Σ 96.2 | 81.2*↑ | Σ 94.5 | 60.7 | Σ 93.8 | 31.5 | Σ 80.7 |
| 12 | 31.7 | | 15.1 | | 13.3 | | 33.1 | | 49.2 | |
| > 12 | 5.5 | | 3.8 | | 5.5 | | 6.2 | | 19.3 | |
| Fur growth (scheduled day 13) | | | | | | | | | | |
| < 14 | 92.1 | Σ 100 | 94.3 | Σ 100 | 90.3 | Σ 100 | 79.8*↓ | Σ 100 | 36.5 | Σ 80.2 |
| 14 | 7.9 | | 5.7 | | 9.7 | | 20.2 | | 43.7 | |
| > 14 | 0 | | 0 | | 0 | | 0 | | 19.8 | |
| Ears opening (scheduled day 13) | | | | | | | | | | |
| < 14 | 68.3 | Σ 98.8 | 80.5*↑ | Σ 100 | 80.0*↑ | Σ 98.2 | 70.8 | Σ 99.5 | 47.7 | Σ 89.3 |
| 14 | 30.5 | | 19.5 | | 18.2 | | 28.7 | | 41.6 | |
| > 14 | 1.2 | | 0 | | 1.8 | | 0.6 | | 10.7 | |
| Eyes opening (scheduled day 15) | | | | | | | | | | |
| < 16 | 18.4 | Σ 66.3 | 19.5 | Σ 76.1 | 16.4 | Σ 72.2 | 6.2*↓ | Σ 73.6 | 20.8 | Σ 80.7 |
| 16 | 47.9 | | 56.6 | | 55.8 | | 67.4 | | 59.9 | |
| > 16 | 33.7 | | 23.9 | | 27.9 | | 26.4 | | 19.3 | |
| Correct running (scheduled day 13) | | | | | | | | | | |
| < 14 | 50.3 | Σ 86.5 | 69.8*↑ | Σ 93.7 | 75.8*↑ | Σ 96.4 | 72.5*↑ | Σ 93.8 | 53.8 | Σ 85.3 |
| 14 | 36.2 | | 23.9 | | 20.6 | | 21.3 | | 31.5 | |
| > 14 | 13.5 | | 6.3 | | 3.6 | | 6.2 | | 14.7 | |
| Descensus testis (scheduled day 21, calculation based on day 22) | | | | | | | | | | |
| < 22 | 2.6 | Σ 21.0 | 13.9 | Σ 19.5 | 26.3*↑ | Σ 31.6 | 10.0 | Σ 15.0 | 14.0 | Σ 60.5 |
| 22 | 18.4 | | 5.6 | | 5.3 | | 5.0 | | 46.5 | |
| > 22 | 78.9 | | 80.6 | | 68.4 | | 85.0 | | 39.5 | |
| Descensus testis (scheduled day 21, for technical reason calculation based on day 24 is filed in the raw data) | | | | | | | | | | |
| < 24 | 55.3 | Σ 57.9 | 38.9 | Σ 66.7 | 68.4 | Σ 73.7 | 40.0 | Σ 67.5 | | |
| 24 | 2.6 | | 27.8 | | 5.3 | | 27.5 | | | |
| > 24 | 42.1 | | 33.3 | | 26.3 | | 32.5 | | | |
| Vaginal opening (scheduled day 36) | | | | | | | | | | |
| n litters | 19 | | 18 | | 18 | | 20 | | 21 | |
| < 37 | 43.2 | Σ 70.2 | 41.7 | Σ 63.9 | 52.8 | Σ 72.2 | 45.0 | Σ 70.0 | 73.8 | Σ 85.7 |
| 37 | 27.0 | | 22.2 | | 19.4 | | 25.0 | | 11.9 | |
| > 37 | 29.7 | | 36.1 | | 27.8 | | 30.0 | | 14.3 | |
| Preputial separation (scheduled day 44) | | | | | | | | | | |
| n litters | 19 | | 18 | | 19 | | 20 | | 22 | |
| < 45 | 28.9 | Σ 68.4 | 44.4 | Σ 69.4 | 39.5 | Σ 73.7 | 17.5 | Σ 50.0 | 81.4 | Σ 90.7 |
| 45 | 39.5 | | 25.0 | | 34.2 | | 32.5 | | 9.3 | |
| > 45 | 31.6 | | 30.6 | | 26.3 | | 50.0 | | 9.3 | |

* significant difference ($p<0.05$); ↑ = increased ; ↓ = decreased ; Σ sum of frequencies of animals showing the respective postnatal physical parameter on the scheduled day and one day later

**Neurological Assessment –** NOAEL = 30 mg/kg/d. Reflexes (pupillary reflex, air-righting reflex) and sensory functions (hearing, Preyer reflex) were not affected by treatment. Learning endpoints (Biel water T-maze test) were not affected by treatment in week 6. Memory as measured in the same water test during week 7 showed a delay in HD males (ss) and a potential trend in HD females (nss). The data showed many of the affected animals were below average BW compared to littermates and/or controls. The trend for learning delays correlating with lower BW was not as clear as the correlation between delayed growth and delayed physical development. Because memory/learning delays were not clearly correlated with BW and delayed development across treatment groups, the HD delays in learning were considered drug related. There were no differences in learning in water tests at the end of week 7, suggesting learning delays were not permanent or severe.

Spontaneous activity and exploratory behavior were assessed in shuttle boxes representing unfamiliar environment. There was no clear dose-related effect to suggest marked changes in activity or exploratory behavior. HD males and females did show decreased upper frame activity at 15 min and 30 min time points, suggesting a treatment effect on exploratory behavior of the upper portion of the unfamiliar environment. For activity in the lower frame, or ground level, there were no biologically significant effects in males but there were statistically significant decreases in female behavior in all groups by the end of the time period (i.e., only at the 60 min time point). Because the lower frame findings at 60 min occurred in all female drug-treated groups the findings were not attributed to lower body weights (since only HD groups had lower body weights compared to controls). The absence of an effect in males coupled with the absence of a dose-related effect in females and behavioral changes only at the 60 min time point, biological significance of slightly decreased lower frame activity is unlikely. Effects on behavior considered potentially biologically significant are summarized in the Reviewer's table, below.

**Spontaneous activity and exploratory behavior †**

| Behavior/Activity | Sex | Time Point [a] | |
|---|---|---|---|
| | | 0-15 min | 15-30 min |
| Total activity (Upper Frame) | Male | 300 mg/kg | 300 mg/kg |
| | Female | 300 mg/kg | 300 mg/kg |
| | Combined | 300 mg/kg | 300 mg/kg |
| Center region (Upper Frame) | Male | NS | NS |
| | Female | 300 mg/kg | NS |
| | Combined | 300 mg/kg | NS |
| Border regions (Upper Frame) | Male | 300 mg/kg | 300 mg/kg |
| | Female | 300 mg/kg | 300 mg/kg |
| | Combined | 300 mg/kg | 300 mg/kg |

† Activity measured for one hour in 15 min increments based on activity in lower (ground level) or upper (exploration above ground level, e.g., walls, hind-leg rears) frames

[a] Dose at which statistically significant findings were considered potentially biologically significant during each time interval

NS – considered not biologically significant by the FDA reviewer

**Reproduction –** NOAEL = 30 mg/kg/d. There were no treatment-related effects on pregnancy success or copulation and fertility indices in the mated $F_1$ animals. The majority of $F_1$ animals mated successfully within four days of cohabitation, with no clear treatment-related differences in mating time. Summary data are shown in the Sponsor's tables, below.

**Sponsor's summary tables of $F_1$ fertility and reproductive success**

BI 1356 BS: Study for effects on pre- and postnatal development including maternal function in rats - mating outcome and fertility of the offspring

| Group | Control | 10 mg/kg | 30 mg/kg | 300 mg/kg |
|---|---|---|---|---|
| Sample size (n litters) | 19 | 18 | 18 (19)# | 20 |
| Parameter | | | | |
| Offspring with sperm and pregnant | 16 | 16 | 16 (17)# | 19 |
| Offspring without sperm but pregnant | 0 | 1 | 0 | 0 |
| Offspring with sperm and non-pregnant | 2 | 0 | 1 | 0 |
| Offspring without sperm and non-pregnant | 1 | 1 | 1 | 1 |
| Frequency (%) | | | | |
| Copulation index | 94.74 | 88.89 | 94.44 (94.74)# | 95.00 |
| Fertility index | 88.89 | 100.00 | 94.12 (94.44)# | 100.00 |
| Gestation index | 100.00 | 100.00 | 100.00 | 100.00 |

# male No. 312/7 included due to one litter (307) with males only

BI 1356 BS: Study for effects on pre- and postnatal development including maternal function in rats - day after start of mating when sperm was found in the vaginal smear

| Group | Control | 10 mg/kg | 30 mg/kg | 300 mg/kg |
|---|---|---|---|---|
| Sample size (n litters) | 19 | 18 | 18 (19)## | 20 |
| n/frequency [%] decimals truncated | | | | |
| Day after start of mating | | | | |
| 1 | 4 (21.05) | 8 (44.44) | 6 (33.33) | 5 (25.00) |
| 2 | 4 (21.05) | 3 (16.66) | 5 (27.77) | 5 (25.00) |
| 3 | 5 (26.31) | 3 (16.66) | 2 (11.11) | 6 (30.00) |
| 4 | 5 (26.31) | 2 (11.11) | 4 (22.22) | 3 (15.00) |
| | | | | |
| Sperm found but not pregnant | 2 (11.11)# | 0 | 1 (5.88)# | 0 |
| No sperm found but pregnant | 0 | 1 (5.55) | 0 | 0 |
| Not successfully mated (no sperm found, non-pregnant) | | | | |
| After 10 days of mating | 1 (5.26) | 1 (5.55) | 1 (5.55) | 1 (5.00) |

\# sample size (n sperm found) =18 in the control group and 17 in the 30 mg/kg dose group
\## Due to one litter (307) in the 30 mg/kg dose group with males only, male No. 312/7 was mated with the remaining female (No. 301/11) from the same dose group. This female was pregnant (sperm found after 2 days of mating) but was not included into further evaluation.

There were slight decreases in the reproductive success of the HD $F_1$ generation. There were slight but statistically significant decreases in the number of viable fetuses ($F_2$) in the HD group (10.5 vs. 11.9 controls). The numbers of fetuses were within the range of concurrent controls and representative historical control ranges (represented by data from three prior studies in the conducting lab). There were a few other trends that were not statistically significant in the HD group that further suggested a slight decrease in reproductive success. The number of corpora lutea (12.5 vs. 13.3 controls, nss) and implantations (11.3 vs. 12.3 controls, nss) were slightly lower in pregnant HD $F_1$ females. In addition, pre-implantation loss (10% vs. 7.3% controls, nss) and resorption rate (7.2% vs. 3.7% controls, nss) were slightly elevated in pregnant HD $F_1$ females. As with the decrease in viable fetus number, the other HD reproductive trends were generally within the range of concurrent and representative historical control ranges.

Testes atrophy (bilateral severe diffuse) was observed histologically in a single HD $F_1$ male whose paired female was not pregnant and had no sperm in the vaginal smear. There were no other histopathology findings in either $F_1$ males or females that failed to reproduce successfully. The isolated incidence of testes atrophy in the HD male was likely spontaneous based on the absence of findings in any other animals.

The slight reduction in overall reproductive success in the HD $F_1$ was consistent with the slightly lower body weights that persisted in HD animals throughout the study. The biological significance of the slightly lower reproductive success rate was considered marginal by this reviewer based on the overall similarity of findings to the variability in control groups and historical control data. Summary data are shown in the Sponsor's tables, below.

**Sponsor's summary table of $F_1$ reproduction parameters**

BI 1356 BS: Study for effects on pre- and postnatal development including maternal function in rats – litter parameters of the offspring

| Groups | G1: Control | G2: 10 mg/kg | G3: 30 mg/kg | G4: 300 mg/kg | Control group from [U06-1586] | Spontaneous incidences from [U04-1440] | Spontaneous incidences from [U05-1493] |
|---|---|---|---|---|---|---|---|
| n litters + | 16 | 17 | 16 | 19 | 20 | 90 | 92 |
| Litter parameters | | | | | | | |
| | means<br>ranges of individual data | | | | | means<br>ranges of means/<br>ranges of individual data | |
| Corpora lutea | 13.3 | 13.1 | 13.2 | 12.5 | 12.3 | 12.3 | 12.1 |
| | 10-17 | 11-16 | 11-17 | 10-16 | 10-16 | 11.9-12.5/<br>8-16 | 11.6-12.5/<br>6-16# |
| Implantations | 12.3 | 12.4 | 12.6 | 11.3 | 11.4 | 11.3 | 11.3 |
| | 7-17 | 11-14 | 11-16 | 7-15 | 6-16 | 11.0-11.6/<br>3-15 | 10.9-11.5/<br>3(3)-16 |
| Viable fetuses | 11.9 | 11.5 | 12.1 | 10.5*↓ | 10.5 | 10.5 | 10.3 |
| | 7-16 | 9-14 | 11-16 | 6-14 | 6-14 | 10.1-11.0/<br>3-14 | 10.1-10.4/<br>1(3)-14 |
| Dead fetuses | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total resorptions | 0.44 | 0.82 | 0.50 | 0.79 | 0.95 | 0.83 | 0.96 |
| | 0-1 | 0[1]-4 | 0[1]-2 | 0[1]-2 | 0[1]-3 | 0.59-1.15/<br>0[1]-3 | 0.79-1.24/<br>0[1]-5 |
| Early resorptions | 0.38 | 0.71 | 0.50 | 0.79 | 0.80 | 0.78 | 0.53 |
| | 0-1 | 0[1]-2 | 0[1]-2 | 0[1]-2 | 0[1]-3 | 0.59-1.15/<br>0[1]-3 | 0.42-0.71/<br>0[1]-3 |
| Late resorptions | 0.06 | 0.12 | 0 | 0 | 0.15 | 0.06 | 0.42 |
| | 0-1 | 0-2 | 0 | 0 | 0-1 | 0-0.17/<br>0-1 | 0.38-0.52/<br>0[1]-3 |
| Pre-implantation loss [%] | 7.30 | 5.58 | 4.45 | 10.05 | 7.24 | 8.01 | 7.24 |
| | 0[7.14]-46.15 | 0[7.14]-21.43 | 0[7.14]-23.53 | 0[7.14]-46.15 | 0[7.14]-30.00 (53.85) | 7.06-9.50/<br>0[6.25]-57.14 (66.67) | 5.55-9.07/<br>0[7.14]-27.27(100) |
| Resorption rate [%] | 3.73 | 6.57 | 3.84 | 7.23 | 8.32 | 7.71 | 9.00 |
| | 0[5.88]-11.11 | 0[7.14]-30.77 | 0[7.69]-14.29 | 0[6.67]-20.00 | 0[7.14]-18.18 (30.00) | 4.88-11.28/<br>0[6.67]-22.22 (50.0) | 7.40-10.65/<br>0[7.14]-33.33(66.67) |

+ non-pregnant animals excluded , [ ] the lowest number above 0 , ( ) outlyer included , # dam No. 320 with 22 corpora lutea but no implantations excluded, * significant difference (p<0.05) , ↓ decreased

**Toxicokinetics –** Drug exposure was not assessed in the $F_1$ generation. A separate TK study in pregnant female rats showed fetal exposures of approximately 50% the maternal plasma exposure.

**$F_2$ Generation**

**Survival/Mortality –** As noted above, there were slight but statistically significant decreases in the number of viable fetuses ($F_2$) in the HD group (10.5 vs. 11.9 controls). The numbers of fetuses were within the range of concurrent controls and representative historical control ranges (represented by data from three prior studies in the conducting lab).

# 10 Special Toxicology Studies

**Local tolerance studies in rat and rabbit and irritation in rabbit**
*GLP studies*

**Summary:** Several GLP studies were conducted to assess potential immune-mediated and irritation reactions to linagliptin exposure. While some DPP4 inhibitors have caused necrotizing skin lesions in animals, linagliptin caused facial flushing/swelling and reddening in dogs and minipigs but the reactions were not consistent with necrotic skin lesions. Study findings are summarized below. Additional single and multiple dose *iv* injection toxicity studies were conducted to assess tolerability of *iv* infusions. Drug was tolerated for up to two weeks of *iv* infusion and histopathology examination found no drug-related injection site toxicity.

<u>Single dose paravenous tolerance study in rats</u>

**Key study findings:**
- Subcutaneous paravenous injection of BI 1356 BS elicited a local inflammatory reaction characterized by minimal to slight focal mononuclear cell infiltrate in subcutaneous tissues within 24 hours of dosing that resolved by 96 hours post-dose.
- Ninety-six hours after dosing, slight acute hemorrhage occurred at the BI 1356 BS injection site of one rat.

<u>Single dose intra-arterial tolerance study in rabbits</u>

**Key study findings:**
- Ninety-six hours after treatment, BI 1356 BS injection site reactions in rabbit ear arteries were characterized by inflammatory cell infiltrate, focal blood vessel wall damage (fibrosis in arterial wall or necrosis in a vein wall), and in one rabbit, minimal exudation of fibrin. These necropsy findings were not present in 2 rabbits sacrificed 24 hours post-injection.

<u>Single dose intravenous and intramuscular tolerance study in rabbits</u>

**Key study findings:**
- Two female rabbits injected intravenously and intramuscularly with 0.5 ml of 0.5 mg/ml BI 1356 BS (right side) and vehicle (left side) sacrificed 24 h post-injection had treatment-related local damage to blood vessel walls and necrotizing lesions of the skeletal muscle. No treatment-related histopathology findings occurred in 2 rabbits sacrificed 96 hours after treatment.

Acute dermal irritation / corrosion study in rabbits

**Key study findings:**

- 0.5 g BI 1356 BS paste topically applied to 6 cm2 bare rabbit dorsal skin for up to 4 hours was not irritating or corrosive.

**Hemolysis of human blood *in vitro***

*GLP statement, 1/22/07*

**Hemolysis test with an injectable solution of BI 1356 BS (0.5 mg/ml, calculated as base) and place. Amendment 1 (06B045 A1; Doc. No. U06-1761-01)**

**Key Study Findings:**

- Linagliptin was negative for hemolysis of human blood cells *in vitro*

**Summary:** Serial dilutions of a standard injectable solution of BI 1356 BS for human *iv* administration were incubated *in vitro* (37°C, 45 min) with blood from three different human donors. Hemolytic potential was estimated by absorbance at 570 nm by comparing drug samples with solvent control (0.9% NaCl) and positive control (1% saponine in saline) which were expected to represent 0% and 100% hemolysis, respectively. BI 1356 BS was negative for hemolytic activity with negligible hemolysis detected (< 0.2%).

(b) (4)



1 Page Withheld as B4 (CCI/TS) Immediately Following this Page

**Monkey 2-week *iv* infusion**
*GLP assay, 2/9/10*

**Toxicity study by intravenous infusion administration to cynomolgus monkeys for 2 weeks (Study BOI0322/053412; Doc. No. U10-1202-01)**
*0, 1, 5, 40 mg/kg/d*
*1835, 9690, 101850 nmol*h/l*

*NOAEL = 5 mg/kg (82X MRHD)*

**Summary:** Monkeys (3/sex/group) were administered 0, 1, 5, or 40 mg/kg linagliptin by *iv* infusion (10 min) for two weeks. Findings in HD included clinical signs of decreased activity and pseudoallergy-type hypersensitivity (with increased plasma histamine), dose-related decreased BW in HD males, increased 2.6-fold ALT in one HD female, and cardiovascular findings (PR and QRS prolongation, ↓ systolic BP). ***NOAEL = 5 mg/kg; HD toxicity occurred at greater than 600-times the expected MRHD.***

**Key Study Findings:**

- HD male clinical signs (facial reddening and swollen lips, muzzle, and groin) and increased plasma histamine indicative of pseudoallergy-type hypersensitivity reaction
- HD cardiovascular effects of PR and QRS prolongation and decreased systolic blood pressure
- Toxicity in the HD occurred at greater than 600-times MRHD

**Linagliptin excretion in milk from lactating rats**
*Non-GLP study, dated 9/23/08*

**Summary:** Drug and metabolite excretion in milk was assessed in lactating female Wistar rats treated with a single 30 mg/kg oral gavage dose of BI 1356. Milk was collected for 15 min at 1, 6, and 24 h postdose and plasma was also collected at 24 h postdose. Oxytocin (1.5 IU) was injected *sc* 5 min prior to milking to enhance collection. Results showed BI 1356 equivalents were rapidly excreted into milk with 4-fold higher levels of drug in milk than plasma at $T_{max}$. Overall, the amount of drug in milk was low compared to the administered dose, ranging from AUC of 0.2% to 0.6% and a mean AUC of 0.35%, of the total administered dose. Total radiolabled BI 1356 equivalents in milk ranged from $AUC_{0-24}$ = 19,100 to 62,900 nmol*h/l (**$\bar{x}$ = 36,760 nmol*h/l**). Parent drug represented approximately 90% to 96% of milk equivalents. Up to 7 metabolites were identified in different milk samples, representing only 0.03% of administered dose and maximum individual metabolites at 0.006% (CD 1790) to 0.008% (M515(1)) of total dose. In plasma, approximately 52% of drug equivalents were found in the non-extractable fraction at 24 h postdose, representing the plasma protein bound fraction. Unmetabolized parent made up 73% of the protein bound fraction.

**Key study findings**:

- Drug and CD 1750 metabolite were rapidly excreted in milk of lactating female rats after oral BI 1356 dosing
- Drug exposure in milk was 4-fold higher than maternal plasma at $T_{max}$
- Total mean milk exposure ($AUC_{0-24}$) of 36,760 nmol*h/ml was 233-times the expected clinical plasma exposure

# 11 Integrated Summary and Safety Evaluation

The proposed linagliptin film-coated tablet was submitted in accordance with 21 USC 505(b)(1) for treatment of type 2 diabetes mellitus. A comprehensive battery of nonclinical studies were conducted to support development of linagliptin for chronic use. Linagliptin is the fifth DPP4 inhibitor application submitted for FDA review of its potential to treat type 2 diabetes. Two DPP4 inhibitors have been approved for diabetes treatment and linagliptin toxicity compares favorably with the listed drugs. All pivotal studies were conducted in compliance with current GLP standards.

Most nonclinical studies were reviewed in the course of drug development and are summarized in the NDA review. Exposure margins have been adjusted to account for the proposed lower clinical exposures.

***Pharmacology***

Linagliptin is a high potency, competitive, reversible inhibitor of DPP4 with an approximate human $IC_{50}$ of 3.6 nM in plasma and similar $IC_{50}$s of 7 to 10 nM in mouse, rat, and dog plasma. Linagliptin has a high affinity for direct DPP4 protein binding. The high affinity DPP4-binding affects linagliptin pharmacokinetics and influences concentration-dependent DPP4 inhibition characteristics *in vivo*. DPP4-specific linagliptin binding saturation occurs at the same order of magnitude as DPP4 inhibition. While protein binding clearly affects pharmacokinetics, oral dosing with linagliptin readily exceeds saturating plasma concentrations and achieves potent, durable plasma DPP4 inhibition *in vivo*.

The major metabolite, CD 1790, did not sufficiently inhibit DPP4 activity to expect efficacy *in vivo*. Linagliptin showed >10,000-fold selectivity for DPP4 inhibition compared to the related DPP8 and DPP9, suggesting no off target inhibition at therapeutic exposures. With the exception of linagliptin inhibition of the structurally related FAP ($IC_{50}$ = 94 nM), neither the parent nor CD 1790 showed off target activity towards other receptors or enzymes. Potential for off target effects on hemolysis or wound healing due to FAP inhibition were investigated in several dedicated studies and linagliptin treatment had no significant *in vivo* effects.

Efficacy for DPP4 inhibition, glucose lowering potential, and limited increased insulin sensitivity were confirmed after oral linagliptin treatment in animal models.

Linagliptin showed low potency inhibition of CYP3A4, P-gp, MAO-B, and MDR1, but potential for *in vivo* drug-drug interactions is considered minimal based on very high potency DPP4 inhibition by linagliptin. Linagliptin metabolism and Pg-p mediated transport may be altered with concomitant treatment with CYP3A4 or Pg-p inhibitors.

***PK/ADME***

Linagliptin is rapidly absorbed and distributed after oral dosing. Plasma concentrations rapidly reach levels that inhibit DPP4 by >80% ($T_{max}$ ≈ 30 min), which leads to increased post-prandial incretins and blood glucose lowering. Plasma $t_{1/2}$ were high in all species, ranging from 10 h to >100 h depending on dose and species. Variability in $t_{1/2}$ between low and high doses was evident in different species, with shorter $t_{1/2}$ in higher dose treatments. Tissue accumulation was seen in all species, consistent with DPP4 tissue distribution. The high tissue distribution and variable $t_{1/2}$ were attributable to saturable, high affinity binding to DPP4 in plasma and tissues. After saturation of plasma DPP4, which occurs at slightly higher concentrations than the calculated plasma $IC_{50}$ values, linear PK trends occur, tissue distribution stabilizes as tissue DPP4 is saturated, and linagliptin is more readily eliminated resulting in shorter $t_{1/2}$ compared to non-saturating conditions. DPP4-specific binding was confirmed in investigative studies with DPP4 knockout mice and DPP4-deficient rats.

Linagliptin is not extensively metabolized in animals or humans. The majority of administered linagliptin is excreted unchanged in feces. A single major metabolite, CD 1790, was identified in humans (13% administered dose) and animals. The qualitative pattern of linagliptin metabolism is similar across species and CD 1790 toxicity was adequately evaluated in mouse, rat, rabbit, and monkey toxicity studies. Neither CD 1790 nor any other metabolites were found to be pharmacologically active *in vitro* based on DPP4 inhibition.

***Toxicology***

Linagliptin was generally well tolerated in healthy and diabetic animals. No major toxicity issues were previously identified at End of Phase 2 or pre-NDA meetings with the Sponsor. Irreversible and/or non-monitorable toxicity typically occurred only at very high exposure multiples (> 50-times the MRHD). Linagliptin did not cause skin lesions in monkeys, which has been the hallmark toxicity for some drugs in the DPP4 inhibitor class. A pseudoallergy-type, hypersensitivity response was seen in dogs and minipigs after oral dosing and in monkeys only after *iv* dosing at extremely high exposure multiples (>600X MRHD). The toxicity was evident in clinical signs of facial flushing/reddening and edema, but high dose of linagliptin were tolerated without other dose-limiting toxicity. The pseudoallergy reactions were shown to involve systemic histamine release but there was no evidence of an IgE-mediated allergic response that could lead to anaphylaxis. Investigative studies showed linagliptin was not an irritant or hemolytic. Some studies showed a modest local inflammatory response to linagliptin injection but no drug-related injection site toxicity was observed in multiple dose *iv* studies in monkeys.

Target organs were identified only at very high exposure multiples with NOAELs >30-times clinical exposure in chronic toxicity studies. Kidney, liver, lung, stomach, and testes toxicity occurred at >90-times the MRHD. Toxicities included: kidney tubular degeneration, necrosis/apoptosis, and increased plasma and urine biomarkers; liver increased organ weight and cytoplasmic rarefaction (glycogen accumulation), centrilobular hypertrophy, and plasma ALT biomarker; stomach erosion and necrosis; testes gross changes (decreased size, prominent tubules) and germ cell depletion, mineralization, and epididymal dilatation; and, lung increased alveolar macrophages suggestive of phospholipidosis. Toxicity suggestive of phospholipidosis in lung was seen in short term rat studies and chronic lifetime treatment with >400-times the MRHD caused lung cholesterol cleft granuloma(ta). Overall cholesterol cleft incidence was modest in rat HD males (27%) and females (27%) but trends in both sexes showed a drug-related HD increase compared to concurrent controls. Lesions suggesting drug-related lung phospholipidosis (e.g., foamy cell macrophages) in shorter duration rat studies are consistent with cholesterol cleft granuloma(ta) as secondary to lung phospholipidosis after chronic treatment. The biological significance of rat chronic lung phospholipid findings is unclear based on the absence of a significant toxicological correlate (e.g., increased clinical signs, mortality, or evidence of progression to neoplasms). Clinical risks of phospholipidosis are considered minimal based on findings only at very high exposure multiples.

Pancreatitis is currently being monitored for sitagliptin and it has been identified as a safety issue for clinical monitoring for other DPP inhibitors and GLP-1 mimetics and protein/peptide analogs. No nonclinical signal for pancreatitis was seen in toxicity studies with standard healthy animal models. Pancreatitis may not be adequately assessed in healthy animals but there are currently no good animal models to investigate drug-induced pancreatitis. Nevertheless, there were no nonclinical findings to suggest any increased risk of pancreatitis even after chronic linagliptin exposure.

Developmental and reproductive toxicity were assessed in a standard battery of assays. There were no apparent effects of treatment on fertility in either male or female rats treated prior to mating with estimated exposures >800-times the MRHD. Neither reproductive nor embryofetal toxicity were evident in pregnant rats in the fertility study. Embryofetal development was investigated thoroughly in both rats and rabbits. Maternal toxicity was evident in HD rats (1000X MRHD) based on reduced BW and BW gain. Rat HD linagliptin also resulted in slightly (nss) decreased number of corpora lutea, embryofetal survival and increased late resorptions. Various skeletal variations, generally associated with delayed ossification, were increased across treatment groups but were not clearly associated with or predictive of skeletal malformations. There were slight increases in skeletal malformations above concurrent controls but the final NOAEL considered LD and MD findings to not be treatment related based on absence of a clear dose-response, historical control findings, and absence of any malformations in MD above the historical range. Rib malformations (flat and thickened) in HD were only slightly outside the historical control range (~+4%), but they were considered potentially drug-related because a 2.5-fold higher dose caused a very high incidence (57% incidence) of the same rib malformations in a range-finding assay. The conducting laboratory apparently does not track control group findings across multiple studies. Rather, “historical control” data are reported from dedicated studies with large numbers of vehicle treated controls. In addition, control data from individual studies were included for reference. The absence of ongoing historical control data is unorthodox but in the case of fetal variations and malformations in rats, the totality of control data showed rat variations and malformations were not remarkable and not indicative of any clear teratogenic effect of linagliptin. Rabbit embryofetal toxicity trends also did not show any teratogenic potential of linagliptin up to very high clinical exposure multiples. Rabbit maternal and embryofetal toxicity were evident in the HD (1943X MRHD) based on increased resorptions and decreased BW gain concomitant with decreased food consumption. There were no treatment-related fetal rabbit malformations. Fetal variations of small gall bladder/hypoplasia and increased lumbar ribs (summa) were increased in LD and HD treatment groups compared to concurrent and historical controls. The absence of a dose-response due to absence of similar variations in the MD, coupled with the relatively common occurrence of gall bladder abnormalities in fetal rabbits, suggest LD findings may be incidental and not biologically significant.

A separate rat embryofetal development study was conducted for linagliptin in combination with metformin to support a separate fixed dose combination linagliptin plus metformin NDA. A cursory review of the data, with particular emphasis on types and incidence of fetal variations and malformations, showed a 5 mg/kg linagliptin only dose was the NOAEL. The absence of findings in the 5 mg/kg linagliptin group provided further support that fetal variations and malformations seen in the LD (10 mg/kg) but not the MD (30 mg/kg) rat were incidental and not drug related.

Rat treatment during pregnancy ($F_0$) and *in utero* and throughout lactation ($F_1$) with 300 mg/kg linagliptin (> 1000X MRHD) resulted in delayed growth and development of offspring in the pre- and postnatal reproductive toxicity study. Maternal toxicity was evident at the HD based on decreased maternal BW gain and BW compared to controls. Delayed labor and postimplantation loss were increased in the HD dams. Body weights of offspring ($F_1$) from HD dams were decreased at birth and remained lower than controls throughout adolescence, mating, and pregnancy. HD offspring ($F_1$) also had modest delays in physical development, learning/memory, physical activity/exploratory behavior, and in number of viable offspring ($F_2$) after mating. Mating, fertility, and pregnancy of $F_1$ animals were generally otherwise unaffected by the *in utero* and lactational exposure to the HD of drug. Fetal and neonatal exposure were not measured in this study but separate studies confirmed linagliptin crosses the placenta to expose developing fetuses and it is excreted in milk at 4-times the concentration of maternal plasma. The 30 mg/kg NOAEL for maternal and $F_1$ findings provided approximately 49-fold (maternal) and 24-fold ($F_1$) exposures based on actual AUC in dams and estimated 50% exposure in fetuses and unknown continued $F_1$ exposure during nursing.

Maternal transfer of linagliptin and metabolite CD 1750 were confirmed in rat and rabbit through placental transfer and nursing rat pups were exposure to linagliptin excreted in milk. Linagliptin in milk was four times more concentrated than linagliptin in plasma. Neonatal exposure is assumed based on good overall oral bioavailability and the absence of evidence that linagliptin would be retained in milk and not absorbed in nursing pups. There were species differences in fetal exposure, with rat fetuses exposed to up to 50% of total maternal plasma exposure and 40% of maximal maternal plasma levels. Rabbit fetal exposure was much lower compared to mothers, with only 4-5% of maternal plasma maximum and total maternal exposure.

Male and female reproductive tissue toxicity was seen at very high multiples of clinical exposure in chronic mouse and monkey studies. Lifetime exposure to mice in the carcinogenicity study resulted in HD male (>200X MRHD) findings of gross increased testes 'prominent tubules', plus histologic increased severity of germ cell depletion, increased incidence and severity of testes mineralization, and epididymid increased duct dilatation, slightly increased epithelial dilatation and decreased numbers of spermatozoa. Male and female monkey sexual maturation were potentially delayed at HD chronic exposure (~800X MRHD), with evidence of decreased prostate weights and trends of decreased testes and epididymis weights. Female monkey uterine and ovary weights were slightly decreased after chronic exposure (≥40X MRHD) compared to concurrent controls but weights were generally within the historical range. NOAELs for reproductive tissue toxicity were established in all studies, providing large exposure multiples (NOAELs >30X MRHD). As noted previously, shorter duration exposures did not affect rat fertility or reproductive success of rats exposure *in utero* and throughout lactation, further suggesting minimal clinical risk of reproductive tissue toxicity. Overall, no specific risks from reproductive toxicity studies are predicted for fetuses, neonates, or nursing infants at clinical exposures.

Linagliptin carcinogenicity was assessed in standard two-year oral gavage carcinogenicity studies in mice and rats. Doses provided much greater than 25-fold multiples of human exposure for both species and protocols and dosing regimens were considered appropriate by the Division and by the ECAC. Final study reports were considered acceptable to assess carcinogenic potential. Lifetime linagliptin exposure caused drug-induced lymphomas in female mice at 287-times the MRHD. No other drug-related tumors were seen in mice or rats. The biological significance of drug-induced tumors in a single sex of a single species is not clear without further investigation of the mechanism of tumor induction. The NOAEL for drug-related tumors in female mice provided a 34-fold margin over expected human exposures.

Linagliptin poses minimal carcinogenic risk to humans based on high exposure multiples at the NOAEL for tumor incidence (34X) and very high exposure multiples (287X) at the linagliptin dose that caused tumors in a single sex in mice. In addition, no drug-related tumors were found in rats exposed to over 400-times the MRHD.

Linagliptin showed no evidence of genotoxicity in a standard battery of genotoxicity assays (Ames, *in vitro* CHO cell chromosome aberration in human lymphocytes, and *in vivo* repeat dose (4-week) rat micronucleus). Metabolite CD 1750 XX was also negative for mutagenicity (Ames assay) and clastogenicity (*in vitro* HPBL chromosome aberration assay). Several drug substance impurities required qualification based on specifications exceeding the qualification threshold. Several additional 'potential' impurities were identified in the drug product. The Sponsor conducted genotoxicity studies for all of the listed and potential impurities. In addition, all of the drug substance impurities were present in all pivotal rat or monkey studies (usually both species) and in the carcinogenicity studies.

The nature of the drug substance formulation results in a long list of actual and theoretical impurities. All of the listed organic impurities were qualified for toxicity individually in genetic toxicity assays and *in vivo* in nonclinical toxicology studies. Drug substance impurities were also present in batches used for two year mouse and rat carcinogenicity studies, which showed no evidence of drug-related tumors up to 34- and 418-times the clinical dose in mice and rats, respectively. Overall, all impurities and degradants were qualified in accordance with current guidance and none are considered to pose a significant toxicologic risk.

**Table 15 – Target Organ Toxicity Summary**

| BI 1356 BS – Summary of Major Target Organ Toxicity† | | | | | |
|---|---|---|---|---|---|
| **Organ/Tissue** | **Toxicity** | **LOAEL** | **NOAEL** | **Toxicity Ratio and Safety Margin [a]** | |
| | | **Dose (mg/kg/day) / $AUC_{0-24}$ (µM*h)** | **Dose (mg/kg/day) / $AUC_{0-24}$ (µM*h)** | **LOAEL/ NOAEL** | **NOAEL/ MRHD** |
| Kidney | Proximal tubule basophilia (rat) | 100 mg/kg (6mo) AUC = 55 | 30 mg/kg (6mo) AUC = 10 | 5x | 64x |
| | ↑ weight (rat) | 300mg/kg(≥3 mo) AUC = 282 | 100mg/kg (6mo) AUC = 55 | 5x | 350x |
| | Tubular epithel. vacuolation, hypertrophy, degeneration (rat) | 600 mg/kg (1mo) AUC = 496 | 300mg/kg (6mo) AUC = 282 | 2x | 1800X |
| | Tubule apoptosis/ necrosis (dog) | 45 mg/kg (1mo) AUC = 38 | 9 mg/kg (1mo) AUC = 7 | 5x | 44x |
| | Tubular hyper. basophilia, necrosis, epithel. vacuolat. (mouse) | 600 mg/kg (3mo) AUC = 521 | 300mg/kg (3mo) AUC = 294 | 2x | 1875x |
| | Clin. chem., urine biomarkers, edema… (monkey) | 100 mg/kg (1yr) AUC = 125 | 10 mg/kg (1yr) AUC = 6 | 21x | 40x |
| Liver | Cytoplasmic rarefaction (glycogen accum.) (rat) | 100 mg/kg (6mo) AUC = 55 | 30 mg/kg (6mo) AUC = 10 | 5x | 64x |
| | Centrilobular hypertrophy, ↑ALT (rat) | 100 mg/kg (6mo) AUC = 55 | 30 mg/kg (6mo) AUC = 10 | 5x | 64x |
| | ↑ weight, bile duct hyperplasia (rat) | 300 mg/kg (6mo) AUC = 282 | 100mg/kg (6mo) AUC = 55 | 5x | 350x |
| | Necrosis, vacuolation, foam cells (rat) | ≥600mg/kg (1mo) AUC = 496 | 300mg/kg (6mo) AUC = 282 | 2x | 1800X |
| | Inflammation/ infiltrate (dog) | 45 mg/kg (1mo) AUC = 38 | 9 mg/kg (1mo) AUC = 7 | 5x | 44x |
| | Inflammation/ infiltrate, ↑ bilirubin (monkey) | 300 mg/kg (1mo) AUC = 663 | 150mg/kg (3mo) AUC = 279 | 2x | 1800X |
| Lung (phospholipidosis/ ↑ alveolar mΦ) | Rat(3, 6mo) | 300 mg/kg AUC = 282 | 100mg/kg (6mo) AUC = 55 | 5x | 350x |
| | Rat (2yr) (cholesterol cleft granuloma(ta)) | 60 mg/kg (2 yr) AUC = 66 | 18 mg/kg (2 yr) AUC = 8 | 3x | 51x |

| | | | | | |
|---|---|---|---|---|---|
| | Monkey(3mo) | 150 mg/kg<br>AUC = 279 | 100mg/kg (6mo)<br>AUC = 125 | <2x | 825x |
| | Dog(2wk gross red lungs) | 150 mg/kg<br>AUC = 239 | 45 mg/kg (1mo)<br>AUC = 38 | -- | 242x |
| Hypersensitivity/ Pseudoallergy | Facial reddening/ swelling (dog) | 45 mg/kg (1mo)<br>AUC = 38 | 9 mg/kg (1 mo)<br>AUC= 7 | 5x | 44x |
| | Skin swelling/red (minipig) | 150 mg/kg<br>AUC = 424 | None (single dose study) | -- | <2700x |
| Stomach | Erosion (rat) | 300 mg/kg (6mo)<br>AUC = 282 | 100mg/kg (6mo)<br>AUC = 55 | 5x | 350x |
| | Necrosis (rat) | 600 mg/kg (1mo)<br>AUC = 496 | 300mg/kg (6mo)<br>AUC = 282 | 2x | 1800X |
| Testes | Mouse (2 yr) (Germ cell depletion, mineralization, epidid. dilatation) | 80 mg/kg (2 yr)<br>AUC = 38 | 25 mg/kg (2 yr)<br>AUC = 5 | 3X | 32x |
| Death (mortality/ moribund sacrifice) | **Mouse–** GI, kidney | 600 mg/kg (3mo)<br>AUC = 521 | 300mg/kg (3mo)<br>AUC = 294 | 2x | 1875x |
| | **Rat–** brain hemorrhage, unilateral hydronephrosis | ≥300 mg/kg (3, 6mo)<br>AUC = 282 | 100 mg/kg<br>AUC = 55 | 5x | 350x |
| | **Dog–** myocardial necrosis, red lungs | 150 mg/kg (2wk)<br>AUC = 239 | 45mg/kg (1mo)<br>AUC = 38 | 3x | 242x |
| | **Monkey–** kidney tox (nephrotic syndrome?) | 100 (1 yr)<br>AUC = 125 | 10 mg/kg<br>AUC = 6 | 20x | 40x |
| **Rat** | **NOAEL (2 yr)** | | **18 mg/kg AUC=8** | **--** | **51x** |
| **Mouse** | **NOAEL (2 yr)** | | **25 mg/kg AUC=5** | **--** | **32x** |
| **Dog** | **NOAEL (1 mo)** | | **9 mg/kg AUC=7** | **--** | **44x** |
| **Monkey** | **NOAEL (12 mo)** | | **10 mg/kg AUC=6** | **--** | **40x** |
| **Rat** | **CD 1750 XX NOAEL [b]** | | **30 mg/kg AUC=561** | **--** | **28x** |
| **Monkey** | **CD 1750 XX NOAEL [b]** | | **10 mg/kg AUC=1880** | **--** | **94x** |

† Major target organs shown. Additional findings included: modest effects on male and female reproductive tract organs suggesting potential slight, drug-related delayed maturation (no effects on fertility or early embryonic development); local injection site irritation/inflammation characterized by immune cell infiltration, slight hemorrhage/blood vessel wall damage, but not irritating or corrosive on dermal application

[a] Exposure multiples for toxicity ratio, the multiple of toxicity at LOAEL compared to NOAEL, and safety margin for specific toxicity findings at clinical exposures of $AUC_{0-24}$=158 nM*h ($C_{max}$=11 nM)

[b] CD 1750 XX is the racemic mixture of CD 1790, the major r-enantiomer human metabolite with steady state exposure of CD 1790 $AUC_{0-24}$ = 20 nM*h

48 Pages have been Withheld in Full as B4 (CCI/TS) Immediately Following this Page

/s/

----------------------------------------------------

DAVID B CARLSON
03/07/2011
Pharmtox reviewer recommends approval

TODD M BOURCIER
03/07/2011
pharm/tox supports AP

**Executive CAC**
**Date of Meeting:** January 25, 2011

**Committee:** David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Linda Fossom, Ph.D., DPP, Alternate Member
Todd Bourcier, Ph.D., DMEP, Team Leader
David Carlson, Ph.D., DMEP, Presenting Reviewer

**Author of Minutes:** David Carlson, Ph.D., DMEP

The following information reflects a brief summary of the Committee discussion and its recommendations.

**NDA #:** 201280
**Drug Name:** Linagliptin (Proposed Trade Name – Trajenta®)
**Sponsor:** Boehringer Ingelheim Pharmaceuticals, Inc.

**Background**

<u>Linagliptin Rat Carcinogenicity Study</u>

The final study report of a GLP-compliant, standard two-year oral (gavage) carcinogenicity study in Wistar Han rats was reviewed and results were discussed at a meeting of the Executive Carcinogenicity Assessment Committee (ECAC). The study evaluated doses of 0, 6, 18, and 60 mg/kg/day linagliptin administered in 0.5% hydroxyethylcellulose. The high dose exposure was well in excess of 25-times the exposure at the expected maximum recommended human dose (MRHD). Linagliptin treatment did not have any significant effects on body weight or survival.

**Key study findings**: There were no drug-related neoplasms in males or females at any dose tested. Slight increases in thyroid benign C-cell adenoma in mid-dose males and low-dose females were not statistically significant and the neoplasms were not considered biologically significant or treatment related. NOAEL = 60 mg/kg/day (>400X MRHD).

<u>Linagliptin Mouse Carcinogenicity Study</u>

The final study report of a GLP-compliant, standard two-year oral (gavage) carcinogenicity study in CD-1 mice was reviewed and results were discussed at a meeting of the Executive Carcinogenicity Assessment Committee (ECAC). The study evaluated doses of 0, 8, 25, and 80 mg/kg/day linagliptin administered in 0.5% hydroxyethylcellulose. The high dose exposure was well in excess of 25-times the exposure at the expected maximum recommended human dose (MRHD). Linagliptin treatment did not have any significant effects on body weight or survival.

**Key study findings**: Lymphomas were significantly increased in females administered the high dose (HD) of 80 mg/kg/d. The concurrent control lymphoma incidence was approximately 40% below the low end of the conducting laboratory's historical control range. The incidence of lymphomas in the HD females was clearly higher than other treatment groups, outside the conducting lab's historical control range, and statistically significant for both dose-response trend and pair-wise analyses. The high dose exposure in female mice was more than 200-times the MRHD based on total female exposure ($AUC_{0-24}$). Using a NOAEL of 25 mg/kg/d (MD) for females and 80 mg/kg/d (HD) for males, mouse exposures that did not result in any drug-induced neoplasms were greater than 40-fold (females) and 250-fold (males) higher than expected maximum human exposures. No other neoplasms in mice were statistically significant or considered biologically significant or treatment related.

**Executive CAC Recommendations and Conclusions**:

**Rat:**

- The Committee concurred that the study was acceptable, noting prior Exec CAC concurrence with the protocol.

- The Committee concurred that there were no drug-related neoplasms in rats.

**Mouse:**

- The Committee concurred that the study was acceptable, noting prior Exec CAC concurrence with the protocol.

- The Committee concurred that the increased incidence of lymphomas in females was drug-related at very high multiples of human exposure (>200-times the MRHD).

__________________________

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\ NDA 201280/Division File, DMEP
Todd Bourcier/Team leader, DMEP
David Carlson/Reviewer, DMEP
Raymond Chiang/PM, DMEP
ASeifried, OND IO

---------------------------------------------------------------------------------------------------------
**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**

---------------------------------------------------------------------------------------------------------

/s/

----------------------------------------------------

ADELE S SEIFRIED
01/31/2011

DAVID JACOBSON KRAM
01/31/2011

# PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

**NDA Number:** 201280 **Applicant:** Boehringer Ingelheim **Stamp Date:** 7/2/2010

**Drug Name:** Ondero (linagliptin) **NDA/BLA Type:** 505(b)(1)

On **<u>initial</u>** overview of the NDA/BLA application for RTF:

| | Content Parameter | Yes | No | Comment |
|---|---|---|---|---|
| 1 | Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin? | X | | |
| 2 | Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin? | X | | |
| 3 | Is the pharmacology/toxicology section legible so that substantive review can begin? | X | | |
| 4 | Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)? | X | | |
| 5 | If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA). | X | | Drug substance formulation and batch analyses in nonclinical studies are discussed in the Nonclinical Overview, Section 2.4. |
| 6 | Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route? | X | | |
| 7 | Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations? | X | | Statement included in Toxicology Written Summary, Section 2.6.6. |
| 8 | Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions? | X | | No special studies were requested at the pre-NDA meeting. Previous nonclinical requests seem to be included in the NDA submission. |

# PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

| | Content Parameter | Yes | No | Comment |
|---|---|---|---|---|
| 9 | Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57? | X | | Nonclinical label sections seem generally appropriate and include human exposure multiples. Language in pregnancy Section 8.1, and possibly Nonclinical Tox. Section 13 may need revisions. |
| 10 | Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.) | X | | Several impurities in the drug substance were qualified in rat, monkey, and *in vitro* studies. |
| 11 | Has the applicant addressed any abuse potential issues in the submission? | | | N/A (no known abuse potential). |
| 12 | If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted? | | | N/A (not an OTC application). |

**IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? __Yes______**

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

*See electronic signature page*

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

| Application Type/Number | Submission Type/Number | Submitter Name | Product Name |
|---|---|---|---|
| NDA-201280 | ORIG-1 | BOEHRINGER INGELHEIM PHARMACEUTICALS INC | Linagliptin (BI 1356) Tablets. |

---

**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**

---

/s/

---

DAVID B CARLSON
08/12/2010
NDA is fileable from pharmtox perspective

TODD M BOURCIER
08/12/2010
NDA fileable